Candidemia in a major regional tertiary referral hospital – epidemiology, practice patterns and outcomes

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Abstract

Background: Candidemia is a common cause of nosocomial bloodstream infections, resulting in high morbidity and mortality. This study was conducted to describe the epidemiology, species distribution, antifungal susceptibility patterns and outcomes of candidemia in a large regional tertiary referral hospital.

Methods: A retrospective surveillance study of patients with candidemia was conducted at Singapore General Hospital between July 2012 and December 2015. In addition, incidence densities and species distribution of candidemia episodes were analysed from 2008 to 2015.

Results: In the period of 2012 to 2015, 261 candidemia episodes were identified. The overall incidence was 0.14/1000 inpatient-days. C. glabrata (31.4%), C. tropicalis (29.9%), and C. albicans (23.8%) were most commonly isolated. The incidence of C. glabrata significantly increased from 2008 to 2015 (Coefficient 0.004, confidence interval 0–0.007, p = 0.04). Fluconazole resistance was detected primarily in C. tropicalis (16.7%) and C. glabrata (7.2%). fks mutations were identified in one C. albicans and one C. tropicalis. Candidemia episodes caused by C. tropicalis were more commonly encountered in patients with haematological malignancies (p = 0.01), neutropenia (p < 0.001) and higher SAPS II scores (p = 0.02), while prior exposure to echinocandins was associated with isolation of C. parapsilosis (p = 0.001). Echinocandins (73.3%) were most commonly prescribed as initial treatment. The median (range) time to initial treatment was 1 (0–9) days. The 30-day in-hospital mortality rate was 49.8%. High SAPS II score (Odds ratio, OR 1.08; 95% confidence interval, CI 1.05–1.11) and renal replacement therapy (OR 5.54; CI 2.80–10.97) were independent predictors of mortality, while drain placement (OR 0.44; CI 0.19–0.99) was protective.

Conclusions: Decreasing azole susceptibilities to C. tropicalis and the emergence of echinocandin resistance suggest that susceptibility patterns may no longer be sufficiently predicted by speciation in our institution. Candidemia is associated with poor outcomes. Strategies optimising antifungal therapy, especially in the critically-ill population, should be explored.

Keywords: Candida, Bloodstream infections, Antifungal susceptibility, fks, Mortality
Background

*Candida* species are the leading cause of invasive fungal infections and a common cause of hospital-acquired bloodstream infections [1]. Candidemia has a profound impact on patient outcomes and the burden has increased significantly over the years. The crude mortality is high, ranging from 30–50% [2–4]; while the attributable mortality due to candidemia varied from 15–49% [5, 6]. Increasing reports of antifungal resistance, even in newer agents such as the echinocandins, further escalate the complexity in the management of candidemia [7].

Knowledge of antifungal susceptibility patterns is imperative in the selection of early and appropriate antifungal agents for improved patient outcomes. The variable epidemiology of candidemia, contributed by the geographical and temporal variations in incidence and species distribution [4, 8–10], underscores the continuing need for local surveillance of *Candida* species distribution and susceptibility patterns.

Furthermore, the introduction of new echinocandins into Singapore such as anidulafungin in 2008 and micafungin in 2013, coupled with the exponential increase in echinocandin usage in our institution for the past 5 years, suggest that current susceptibility patterns should be reviewed. A recent study has also reported the emergence of echinocandin resistance in the Asia-Pacific region [11]. The objectives of this study were 1) to investigate the incidence, species distribution and antifungal susceptibilities of candidemia, and 2) to describe the clinical features and outcomes of candidemia in our population.

Methods

Study setting and design

A retrospective surveillance study of patients with candidemia was conducted at Singapore General Hospital (SGH) between July 2012 and December 2015. SGH is the largest acute care hospital (1800 beds) in the country, and covers a wide range of medical and surgical specialties. The hospital is the national/regional referral centre for services such as plastic surgery and burns, renal medicine, nuclear medicine, pathology and haematology. SGH accounts for approximately 25% of the total acute hospital beds in the public sector and 20% of acute beds nationwide.

All adult inpatients (at least 21 years old) with ≥ 1 positive blood culture for *Candida* spp. were included in the study. Each positive *Candida* culture must be accompanied with temporally-related clinical signs and symptoms of infection for inclusion into the study. For each patient, only the first candidemia episode was recorded, unless the positive blood culture was obtained ≥ 30 days (with blood culture clearance and resolution of clinical features of infection of the first episode) or involved a different *Candida* spp. isolated from blood culture obtained ≥ 7 days after the first episode. Episodes involving > 1 *Candida* spp. isolated within 7 days of the first episode, defined as “mixed candidemia”, were regarded as a single episode.

Microbiology and antifungal susceptibility testing

*Candida* spp. were isolated from blood using BD BACTEC™ FX (Becton, Dickinson and Company, Sparks, MD). The species were identified using MALDI Biotyper (BrukerDaltonik GmbH, Germany), morphology studies on cornmeal Tween 80 agar, and API 20C AUX (Biomerieux, Marcy l’Etoile, France). Isolates were stored in Microbank™ storage vials (Pro-Lab Diagnostics, Round Rock, TX, USA) at −70 °C until testing.

Antifungal susceptibility testing was performed using Sensititre YeastOne® YO10 panel (Trek Diagnostics System, West Sussex, England) according to manufacturer’s recommendations. Minimum inhibitory concentrations (MICs) for amphotericin B, anidulafungin, caspofungin, micafungin, fluconazole, voriconazole, itraconazole, posaconazole and flucytosine were recorded. *Candida krusei* (Issatchenkia orientalis) ATCC 6258 and *C. parapsilosis* ATCC 22019 (American Type Culture Collection, Manassas, Virginia) were used as quality controls.

MICs were interpreted according to the current species-specific clinical breakpoints provided by the Clinical and Laboratory Standards Institute (CLSI) M27-S4 document [12]. Where clinical breakpoints were not available, the epidemiological cut-off values (ECV) were used to classify the isolates into wild-type or non-wild-type populations [13–15].

Detection of fks mutations

Isolates classified as intermediate or resistant to echinocandins were tested for the presence of mutations in the fks genes. Hot spots 1 and 2 regions of fks1 and fks2 (for *C. glabrata* only) genes were amplified using polymerase chain reaction (PCR), as described previously [16].

Clinical data collection

Clinical characteristics of patients with candidemia were obtained from inpatient charts and electronic medical records using a standardised case report form. Data extracted included demographics, hospitalisation history (previous hospital stay, previous intensive care unit (ICU) stay, length of hospital stay prior to candidemia), underlying medical conditions and prior exposure to invasive interventions (central lines, urinary catheters, drainage devices, invasive ventilation, dialysis, invasive surgery, total parenteral nutrition) and medical therapy (chemotherapy, immunosuppressive therapy, antibiotics, antifungal agents) within 30 days before the first positive blood culture. Charlson comorbidity index at the time of
admission and Simplified Acute Physiology Score (SAPS) on the day of the first positive blood culture were also recorded. Information on the management of candidemia (choice and duration of antifungal agents) and outcome (in-hospital all-cause mortality within 30 days) were collected.

Data and statistical analyses
To calculate and analyse the incidence of candidemia, the number of candidemia episodes were obtained from the clinical microbiology laboratory computerised database, while inpatient-days were obtained from the hospital administrative database. Incidence data was available from 2008, hence trend analyses were performed for the period from 2008 to 2015. Incidence rates were calculated as the number of candidemia episodes per 1000 inpatient-days. Linear regression was used to determine trends over time in the incidences of candidemia.

Categorical variables were presented as numbers and percentages; and were compared using the $\chi^2$ or Fisher’s exact test, as appropriate. Continuous variables were presented as mean $\pm$ SD or median and range; and were compared using the Student’s t test, Mann–Whitney test, or Kruskal–wallis test, depending on the validity of the normality assumption.

A multivariable logistic regression model was used to identify predictors associated with 30-day mortality. Clinically plausible variables identified in the bivariate analysis were included in the multivariable logistic regression model if $p < 0.1$. Significant factors which may covary were grouped and only one factor from each group was selected for entry into the model. The final model was chosen on the basis of biologic plausibility. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to evaluate the strength of any association. For all calculations, a 2-tailed $p$ value of less than 0.05 was considered to reveal a statistical significant difference. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY).

Results
Incidence and species distribution
From 2012 to 2015, 261 candidemia episodes involving 254 patients and 272 isolates were analysed. Seven patients had two separate episodes each with distinct Candida species, while a patient had a repeated episode involving the same Candida species. The incidence was 0.14 episodes per 1000 inpatient-days during the study period. C. glabrata (82/261, 31.4%), C. tropicalis (78/261, 29.9%), C. albicans (62/261, 23.8%), and C. parapsilosis (36/261, 13.8%) accounted for majority of the episodes. Other species including C. dubliniensis (n = 7), C. krusei (n = 3), C. guilliermondii (Meyerozyma guilliermondii) (n = 1), C. kefyr (Kluyveromyces marxianus) (n = 1), C. haemulonis (n = 1) and C. pseudohaemulonii (n = 1) accounted for the remaining episodes. Of these 261 episodes, 11 (4.2%) were mixed candidemia episodes.

The incidence density and species distribution are displayed in Fig. 1. The overall incidence density was 0.15 (range 0.12–0.18) episodes/1000 inpatient-days and 0.89 (range 0.74–1.05) episodes/1000 admissions from 2008 to 2015. Analysing the incidence densities from 2008 to 2015, we found no significant change in the incidence density of candidemia [Coefficient 0.00009, confidence interval (CI) - 0.007–0.007, $p = 0.98$].
However, we did note that the overall incidence density increased from 0.14 in 2014 to 0.18 episodes/1000 inpatient-days in 2015, suggesting the need for continual monitoring. There was a significant increasing trend in the incidence density of \( C. \) glabrata (Coefficient 0.004, CI 0–0.007, \( p = 0.04 \)), while the incidence densities of the other \( Candida \) spp. remained stable. The proportions of \( C. \) glabrata increased from 11.3% in 2008 to 31.6% in 2015 and that of \( C. \) albicans decreased from 44% in 2008 to 19% in 2015.

**Antifungal susceptibilities**

Antifungal susceptibilities were available for 271 isolates, except for one \( C. \) parapsilosis (Table 1). Among isolates with available clinical breakpoints, overall susceptibility rates were 59.5% (153/257) for fluconazole, 86.9% (152/175) for voriconazole, 99.2% (255/257) for anidulafungin, 98.1% (252/257) for caspofungin and 98.9% (254/257) for micafungin. Using the clinical breakpoints, \( C. \) albicans and \( C. \) parapsilosis retained high susceptibility (>94%) to fluconazole and voriconazole. However, more than 20% of the \( C. \) tropicalis isolates were non-susceptible to fluconazole and voriconazole. The proportions of isolates classified as wild-type (MIC value less than or equals to ECV) for fluconazole, voriconazole, itraconazole and posaconazole were similar among \( C. \) albicans, \( C. \) glabrata and \( C. \) parapsilosis (ranged from 94–100%). Decreased susceptibilities (non wild-type; MIC value greater than ECV) to fluconazole and voriconazole, itraconazole and posaconazole were prominent in \( C. \) tropicalis isolates. Echinocandin resistance was rare, occurring only in three isolates (\( C. \) albicans = 1; \( C. \) tropicalis = 1 and \( C. \) glabrata = 1) when assessed using both clinical breakpoints and ECVs. Most isolates had amphotericin B and flucytosine MICs below ECVs (96–100%), although a number of \( C. \) parapsilosis were classified as non-wild-type (20%). The amphotericin B MICs of these non-wild-type isolates were 2 \( \mu \)g/mL, which were just one dilution above the ECV (1 \( \mu \)g/mL) utilised in this study. Furthermore, the ECV used in this study was derived using the YeastOne\textsuperscript{a} method and is one dilution lower than the ECVs for the ECVs for the other species (2 \( \mu \)g/mL) and the ECV derived from broth dilution methods.

\( fks \) mutations were detected in the echinocandin-resistant \( C. \) albicans (caspofungin MIC 4 \( \mu \)g/mL; anidulafungin MIC 0.25 \( \mu \)g/mL; micafungin MIC 2 \( \mu \)g/mL) and \( C. \) tropicalis (caspofungin MIC 2 \( \mu \)g/mL; anidulafungin MIC 0.5 \( \mu \)g/mL; micafungin 1 \( \mu \)g/mL) isolates. Both isolates harbour a point mutation (S645P in \( C. \) albicans and S80P in \( C. \) tropicalis) in the hotspot 1 region of the \( fks1 \) gene. The two isolates remained susceptible to all other antifungals. Interestingly, \( fks \) mutations were not identified in the \( C. \) glabrata isolate which was resistant (caspofungin MIC \( \geq \) 8 \( \mu \)g/mL; anidulafungin MIC 4 \( \mu \)g/mL; micafungin 4 \( \mu \)g/mL).

**Clinical characteristics**

The clinical characteristics of the candidemia episodes are summarised in Table 2. The median age of patients with candidemia was 65 years and incidence did not differ by gender (52.9% male vs. 47.1% female, \( p = 0.59 \)). The episodes occurred primarily in the medical wards (42.1%), followed by intensive care units (ICUs) (38.3%), surgical wards (19.5%). Patients admitted to haematology-oncology (19.9%), internal medicine (19.5%) and general surgery units (12.3%) encountered the most episodes.

Most of the patients presented with multiple comorbidities (median Charlson score = 5, range 0–15), with many having malignancies (40.6%). Diabetes was also common among these patients (39.5%). Prior antibiotic exposure (90.4%), central venous catheter placement (73.6%), and surgery (65.1%) were common risk factors. A large number of patients were colonised or infected with \( Candida \) at other non-blood sites (45.2%) and had concurrent bacterial infections (48.7%). In addition, it appears that candidemia episodes caused by \( C. \) tropicalis were more commonly encountered in patients with haematological malignancies (\( p = 0.01 \)), neutropenia (\( p < 0.001 \)) and higher SAPS II scores (\( p = 0.02 \)). Exposure to echinocandins was also associated with candidemia episodes caused by \( C. \) parapsilosis (\( p = 0.001 \)).

**Antifungal therapy and outcomes**

Antifungal therapy was initiated in 225 (86.2%) episodes (Table 2). All but six of the 36 patients who did not receive treatment died before blood cultures flagged positive. Treatment was not initiated in four patients as they were conservatively managed. Interestingly, physicians elected not to initiate treatment in the remaining two patients.

Echinocandins were the initial treatment of choice (73.3%), followed by azoles (23.1%). Caspofungin (93.4%) was more commonly used, since it was the only echinocandin in the formulary until anidulafungin’s inclusion in August 2015. Among the patients receiving treatment, 32 (14.2%) were already receiving antifungals as prophylaxis or empiric treatment on the day which cultures were taken. Fluconazole was the onlyazole used as initial treatment of candidemia in our institution. The median (range) time to initial treatment was 1 (0–9) days. Treatment was initiated in 73 (32.4%) patients on day of culture and in 172 (76.4%) patients within two days. The median (range) duration of therapy was 15 (1–140) days.

Patients with candidemia were moderately to severely ill – 57.9% were having severe sepsis and the median (range) SAPS II score was 49 (14–103) at the time of culture. Many of these episodes (38.3%) occurred in
### Table 1 Antifungal susceptibilities of major species of *Candida* isolates

| Antifungal      | MIC<sub>50</sub> (μg/mL) | MIC<sub>90</sub> (μg/mL) | MIC Range (μg/mL) | %S<sup>b</sup> | %SDD/I<sup>b</sup> | %R<sup>b</sup> | %WT<sup>c</sup> |
|-----------------|---------------------------|--------------------------|-------------------|--------------|----------------|-------------|---------------|
| *C. albicans* (n = 62) |                           |                          |                   |              |                |             |               |
| Fluconazole     | 0.5                       | 2                        | ≤0.12→≤256        | 95.2         | 1.6            | 3.2         | 93.5         |
| Itraconazole    | 0.06                      | 0.12                     | ≤0.015→≤16        | –            | –              | –           | 96.7         |
| Posaconazole    | 0.015                     | 0.06                     | ≤0.08→≤8         | –            | –              | –           | 96.7         |
| Voriconazole    | ≤0.008                    | 0.03                     | ≤0.008→≤8        | 93.6         | 3.2            | 3.2         | 93.5         |
| Anidulafungin   | ≤0.015                    | 0.03                     | ≤0.015→0.25      | 100          | 0              | 0           | 98.4         |
| Caspofungin     | 0.03                      | 0.06                     | ≤0.015→4        | 98.4         | 0              | 1.6         | 98.4         |
| Micafungin      | ≤0.008                    | 0.015                    | ≤0.008→2       | 98.4         | 0              | 1.6         | 98.4         |
| Flucytosine     | ≤0.06                     | 0.25                     | ≤0.06→≤64      | –            | –              | –           | 96.7         |
| Amphotericin B  | 0.5                       | 1                        | ≤0.12→1         | –            | –              | –           | 100          |
| *C. glabrata* (n = 82) |                           |                          |                   |              |                |             |               |
| Fluconazole     | 16                        | 32                       | 1→≤256            | 92.8         | 7.2            | –           | 97.6         |
| Itraconazole    | 1                         | 1                        | 0.12→≤16       | –            | –              | –           | 93.9         |
| Posaconazole    | 2                         | 2                        | 0.12→≤8        | –            | –              | –           | 95.1         |
| Voriconazole    | 0.5                       | 2                        | 0.03→≤8        | –            | –              | –           | 97.6         |
| Anidulafungin   | 0.03                      | 0.06                     | ≤0.015→4      | 98.8         | 0              | 1.2         | 98.7         |
| Caspofungin     | 0.03                      | 0.12                     | ≤0.03→≤8       | 96.4         | 2.4            | 1.2         | 96.3         |
| Micafungin      | 0.015                     | 0.015                    | ≤0.008→4       | 98.8         | 0              | 1.2         | 98.7         |
| Flucytosine     | ≤0.06                     | 0.12                     | ≤0.06→0.25     | –            | –              | –           | 100          |
| Amphotericin B  | 1                         | 1                        | 0.25→2        | –            | –              | –           | 100          |
| *C. tropicalis* (n = 78) |                           |                          |                   |              |                |             |               |
| Fluconazole     | 2                         | 64                       | 0.5→≤256        | 78.2         | 5.1            | 16.7        | 84.6         |
| Itraconazole    | 0.25                      | 0.5                      | 0.03→≤16      | –            | –              | –           | 96.1         |
| Posaconazole    | 0.12                      | 0.5                      | 0.03–4        | –            | –              | –           | 98.7         |
| Voriconazole    | 0.12                      | 4                        | ≤0.008→≤8      | 75.6         | 11.5           | 12.8        | 80.8         |
| Anidulafungin   | 0.03                      | 0.12                     | ≤0.015→0.5    | 98.7         | 1.3            | 0           | 98.7         |
| Caspofungin     | 0.03                      | 0.06                     | 0.015–2       | 98.7         | 0              | 1.3         | 98.7         |
| Micafungin      | 0.03                      | 0.03                     | ≤0.008→1      | 98.7         | 0              | 1.3         | 98.7         |
| Flucytosine     | ≤0.06                     | 0.12                     | ≤0.06→0.32    | –            | –              | –           | 96.2         |
| Amphotericin B  | 1                         | 1                        | 0.25–2        | –            | –              | –           | 100          |
| *C. parapsilosis* (n = 35) |                           |                          |                   |              |                |             |               |
| Fluconazole     | 0.5                       | 2                        | 0.25→4        | 97.1         | 2.9            | 0           | 100          |
| Itraconazole    | 0.06                      | 0.06                     | ≤0.015→0.12   | –            | –              | –           | 100          |
| Posaconazole    | 0.03                      | 0.06                     | 0.015→0.12   | –            | –              | –           | 100          |
| Voriconazole    | 0.015                     | 0.03                     | ≤0.008→0.6   | 100          | 0              | 0           | 97.1         |
| Anidulafungin   | 0.5                       | 2                        | 0.12→2        | 100          | 0              | 0           | 100          |
| Caspofungin     | 0.25                      | 0.5                      | 0.06–1        | 100          | 0              | 0           | 100          |
| Micafungin      | 0.5                       | 2                        | 0.12–2        | 100          | 0              | 0           | 100          |
| Flucytosine     | ≤0.06                     | 0.5                      | ≤0.06→1      | –            | –              | –           | 100          |
| Amphotericin B  | 1                         | 2                        | 0.25→2        | –            | –              | –           | 100          |

<sup>s</sup> susceptible, <sup>SDD</sup> susceptible dose-dependent, <sup>I</sup> intermediate, <sup>R</sup> resistant, <sup>WT</sup> wild-type

<sup>a</sup>MICs are only reflected for the predominant species

<sup>b</sup>Susceptibilities were assessed based on CLSI species-specific clinical interpretative breakpoints [12]. Clinical breakpoints are not available for itraconazole, posaconazole, flucytosine and amphotericin B for all species and voriconazole for *C. glabrata*

<sup>c</sup>ECVs were derived from [13, 14] and [15]
Table 2 Clinical characteristics of candidemia episodes

| Demographics       | All       | C. glabrata | C. tropicalis | C. albicans | C. parapsilosis |
|--------------------|-----------|-------------|---------------|-------------|----------------|
| n = 261            | n = 261   | n = 75      | n = 71        | n = 59      | n = 33          |
| Male sex           | 138 (52.9)| 37 (49.3)   | 39 (54.9)     | 32 (54.2)   | 22 (66.7)       |
| Median age (range) | 65 (22–101)| 67 (24–95)  | 63 (28–90)    | 68 (27–101) | 61 (28–86)      |
| Ward type          |           |             |               |             | 0.83           |
| Medical ward       | 110 (42.1)| 30 (40.0)   | 35 (49.3)     | 23 (39.0)   | 14 (42.4)       |
| Surgical ward      | 51 (19.5) | 16 (21.3)   | 10 (14.1)     | 14 (23.7)   | 7 (21.2)        |
| ICU                | 100 (38.3)| 29 (38.7)   | 26 (36.6)     | 22 (37.3)   | 12 (36.4)       |
| Elective admission | 27 (10.3) | 12 (16.0)   | 5 (7.0)       | 5 (8.5)     | 4 (12.1)        |
| Comorbidities      |           |             |               |             | 0.03            |
| Malignancies       | 106 (40.6)| 34 (45.3)   | 29 (40.8)     | 23 (39.0)   | 12 (36.4)       |
| Haematological     | 27 (10.3) | 3 (4.0)     | 13 (18.3)     | 6 (10.2)    | 2 (6.1)         |
| Oncological        | 84 (32.2) | 32 (42.7)   | 17 (23.9)     | 18 (30.5)   | 11 (33.3)       |
| With metastases    | 36 (13.8) | 16 (21.3)   | 11 (15.5)     | 6 (10.2)    | 3 (9.1)         |
| Diabetes           | 103 (39.5)| 31 (41.3)   | 25 (35.2)     | 23 (39.0)   | 12 (36.4)       |
| Chronic renal failure | 67 (25.7)| 17 (22.7)   | 22 (31.0)     | 14 (23.7)   | 8 (24.2)        |
| Hepatobiliary disorders | 58 (22.2)| 17 (22.7)   | 20 (28.2)     | 8 (13.6)    | 8 (24.2)        |
| Myocardial infarction | 43 (16.5)| 10 (13.3)   | 13 (18.3)     | 15 (25.4)   | 1 (3.0)         |
| Cerebrovascular disease | 29 (11.1)| 12 (16.0)   | 8 (11.3)      | 4 (6.8)     | 4 (12.1)        |
| Median (range) Charlson score | 5 (0–15)  | 6 (0–15)    | 5 (0–14)      | 4 (0–12)    | 4 (0–9)         |
| Risk factors       |           |             |               |             | 0.04            |
| Central venous catheter | 192 (73.6)| 47 (62.7)   | 55 (77.5)     | 46 (78.0)   | 26 (78.8)       |
| Drain              | 60 (23.0) | 22 (29.3)   | 14 (19.7)     | 16 (27.1)   | 6 (18.2)        |
| Mechanical ventilation | 111 (42.5)| 26 (34.7)   | 31 (43.7)     | 25 (42.4)   | 16 (48.5)       |
| Total parenteral nutrition | 52 (19.9)| 12 (16.0)   | 13 (18.3)     | 12 (20.3)   | 10 (30.3)       |
| Surgery            | 170 (65.1)| 51 (68.0)   | 44 (66.0)     | 39 (66.1)   | 20 (60.6)       |
| Gastrointestinal surgery | 41 (15.7)| 18 (24.0)   | 5 (7.0)       | 9 (24.3)    | 5 (15.2)        |
| Renal replacement therapy | 85 (32.6)| 16 (21.3)   | 28 (39.4)     | 21 (35.6)   | 12 (36.4)       |
| Antimicrobial therapy | 236 (90.4)| 67 (89.3)   | 66 (95.8)     | 53 (89.8)   | 27 (81.8)       |
| Antifungal therapy  | 51 (19.5) | 13 (17.3)   | 15 (21.1)     | 8 (13.6)    | 11 (33.3)       |
| Azole              | 24 (9.2)  | 5 (6.7)     | 10 (14.1)     | 6 (10.2)    | 2 (6.1)         |
| Echinocandin       | 30 (11.5) | 8 (10.7)    | 6 (8.5)       | 2 (3.4)     | 10 (30.3)       |
| Immunosuppressive therapy | 76 (29.1)| 17 (22.7)   | 28 (39.4)     | 16 (27.1)   | 10 (30.3)       |
| Neutropenia        | 21 (8.0)  | 3 (4.0)     | 13 (18.3)     | 2 (3.4)     | 2 (6.1)         |
| Therapy            |           |             |               |             | 0.04            |
| Primary therapy    |           |             |               |             | 0.01            |
| Echinocandin       | 165 (73.3)| 45 (76.3)   | 49 (81.7)     | 32 (60.4)   | 22 (71.0)       |
| Azole              | 52 (23.1) | 12 (20.3)   | 11 (18.3)     | 17 (32.0)   | 8 (25.8)        |
| Others             | 8 (3.1)   | 2 (3.3)     | 1 (1.7)       | 4 (6.8)     | 1 (3.2)         |
| None               | 36 (13.8) | 16 (21.3)   | 10 (14.1)     | 6 (10.2)    | 2 (6.1)         |
| Median (range) time to primary therapy, days | 1 (0–9) | 2 (0–7) | 1 (0–3) | 2 (0–5) | 1 (0–9) |
| Median (range) duration of therapy, days | 15 (1–140) | 16 (2–61) | 11 (1–96) | 16 (1–140) | 15 (2–47) |
critically-ill patients warded in the ICUs. We also observed that some patients (11.9%), who were initially in the general wards at the time of culture, required admission into the ICU after Candida isolation, suggesting that candidemia episodes can result in severe illness. Mortality occurred in 150 (57.4%) episodes during the admission. The 7-day, 14-day and 30-day in-hospital mortality rates were 28.3%, 39.8%, and 49.8%. The mortality rate was lowest in patients infected with C. glabrata (23.5%) \( (p = 0.03) \). Among the 225 patients who received treatment, the 30-day in-hospital mortality rate was 41.4%, while all but two (94.4%) of the non-treated episodes resulted in death.

**Predictors of mortality**

The characteristics of survivors and non-survivors at 30 days are depicted in Table 3. Based on the multivariable logistic regression model, high SAPS II score (Odds ratio, OR 1.08; 95% confidence interval, CI 1.06–1.11) and renal replacement therapy (OR 4.31; CI 2.24–8.28) were the only factors associated with 30-day mortality. Presence of drains was a protective factor (OR 0.45; CI 0.21–0.94). Mortality occurred rapidly in many of the non-survivors, hence receipt/type of antifungal therapy could not be initiated in this subset of patients. To examine the impact of initial antifungal therapy on 30-day mortality, a separate analysis was performed for candidemia episodes where treatment was administered. Results were similar when non-treated episodes were excluded. High SAPS II score, renal replacement therapy and drains placement were significant factors in the multivariable regression model (Table 4). The choice and timing of initial antifungal therapy was not associated with mortality.

**Discussion**

We report here a comprehensive epidemiological study of candidemia conducted at a large tertiary referral centre, which included the clinical characteristics, antifungal treatment, species distribution, antifungal susceptibilities and outcomes of candidemia. Our study showed that the incidence density of candidemia in our institution has remained fairly stable since 2008. This concurs with the general trend of stability in incidence reported in other developed countries, such as the United States and Europe [2, 17]. A recent study comparing candidemias among sites in Asia indicated that rates in Singapore (0.15 episodes per 1000 patient-days) were comparable with most other Asian countries, with the exception of Taiwan (0.37 per 1000 patient-days) and India (1.24 per 1000 patient-days) [10]. On a more global scale, our rates were lower than those in Italy (0.33 per 1000 patient-days) [18], and Brazil (0.37 per 1000 patient-days) [19]. It appears that the species distribution in our institution is changing. Previous local studies reported a predominance of *C. tropicalis*, a finding commonly observed in tropical regions [10, 20]. We observed an increasing proportion of *C. glabrata* from 11% in 2008 to 31% in 2015, overtaking *C. tropicalis* as the predominant species.
Table 3 Characteristics of survivors vs. non-survivors

|                               | Survivors n = 134 | Non-survivors n = 127 | p     |
|-------------------------------|-------------------|------------------------|-------|
| **Demographics**              |                   |                        |       |
| Male sex                      | 73 (54.5)         | 65 (51.2)              | 0.59  |
| Median age (range)            | 64 (22–95)        | 65 (24–101)            | 0.81  |
| **Ward type**                 |                   |                        |       |
| Medical ward                  | 49 (39.6)         | 44 (34.6)              |       |
| Surgical ward                 | 37 (27.6)         | 14 (11.0)              |       |
| ICU                           | 31 (23.1)         | 69 (54.3)              |       |
| Elective admission            | 14 (10.4)         | 13 (10.2)              | 0.96  |
| **Comorbidities**             |                   |                        |       |
| Malignancies                  | 58 (43.3)         | 48 (51.6)              | 0.37  |
| Diabetes                      | 53 (39.6)         | 50 (39.4)              | 0.97  |
| Chronic renal failure         | 22 (16.4)         | 45 (35.4)              |       |
| Hepatobiliary disorders       | 25 (18.7)         | 33 (26.0)              | 0.16  |
| Myocardial infarction         | 19 (14.2)         | 24 (18.9)              | 0.30  |
| Cerebrovascular disease       | 11 (8.2)          | 18 (14.2)              | 0.13  |
| Median (range) Charlson score | 4 (0–15)          | 5 (0–14)               | 0.09a |
| Median (range) SAPS II score  | 43 (14–82)        | 58 (27–103)            |       |
| **Risk factors**              |                   |                        |       |
| Central venous catheter       | 89 (66.4)         | 103 (81.1)             | 0.007a|
| Drain                         | 37 (27.6)         | 23 (18.1)              | 0.07a |
| Mechanical ventilation        | 47 (35.1)         | 64 (50.4)              | 0.01a |
| Total parenteral nutrition    | 28 (20.9)         | 24 (18.9)              | 0.69  |
| Surgery                       | 81 (60.4)         | 89 (70.1)              | 0.10  |
| Gastrointestinal surgery      | 20 (14.9)         | 21 (16.5)              | 0.72  |
| Renal replacement therapy     | 23 (17.2)         | 62 (48.8)              |       |
| Antimicrobial therapy         | 116 (86.6)        | 120 (94.5)             | 0.30  |
| Antifungal therapy            | 27 (20.1)         | 24 (18.9)              | 0.79  |
| Immunosuppressive therapy     | 33 (24.6)         | 43 (33.9)              | 0.10  |
| Neutropenia                   | 10 (7.5)          | 11 (8.7)               | 0.72  |
| **Therapy**                   |                   |                        |       |
| Initial therapy               |                   |                        |       |
| Echinocandin                  | 89 (66.4)         | 76 (59.8)              |       |
| Azole                         | 40 (29.9)         | 12 (9.4)               |       |
| Others (Amphotericin or combination) | 3 (2.2) | 5 (3.9) |       |
| None                          | 2 (1.5)           | 34 (26.8)              |       |
| Received initial therapy within 24 h | 58 (43.2) | 64 (50.4) |       |
| **Infection Characteristics** |                   |                        |       |
| **Species**                   |                   |                        |       |
| C. albicans                   | 32 (23.9)         | 27 (21.3)              |       |
| C. glabrata                   | 39 (29.1)         | 36 (28.3)              |       |
| C. tropicalis                 | 29 (21.6)         | 42 (33.1)              |       |
| C. parapsilosis               | 24 (17.9)         | 9 (7.1)                |       |
Table 3 Characteristics of survivors vs. non-survivors (Continued)

| Characteristics                                      | Survivors | Non-survivors | p-value |
|-------------------------------------------------------|-----------|---------------|---------|
| Median (range) time to reporting positive culture, days| 2 (0–10)  | 2 (0–10)      | 0.08a   |
| Median (range) time to species identification, days   | 5 (2–16)  | 5 (2–22)      | <0.001a |
| Median (range) Candida score                          | 2 (0–5)   | 3 (0–5)       | 0.01    |
| Median (range) Pitts’ bacteraemia score               | 2 (0–11)  | 5 (0–14)      | <0.001a |
| Severe sepsis at time of culture                      | 64 (47.8) | 87 (68.5)     | <0.001a |
| Concurrent bacterial infection                        | 59 (46.5) | 68 (53.5)     | 0.12    |
| Candida colonization/infection at other sites         | 61 (45.5) | 57 (44.9)     | 0.92    |

All variables are denoted as number of patients with the characteristic or belong to the category n (%), unless otherwise stated. Significant variables are reflected in bold and italics.

aAdditional factors entered into multivariable logistic regression model including only treated episodes.

With respect to antifungal susceptibilities, while *C. albicans* and *C. parapsilosis* remained mostly susceptible, fluconazole resistant rates of *C. tropicalis* was 17%. Notably, the fluconazole MIC90 of *C. tropicalis* increased from 2 μg/mL in 2007 to 64 μg/mL reported in our study [20]. This MIC uptrend suggests that *C. tropicalis*, one of the predominant species in our context, is increasingly becoming less susceptible. Further molecular investigations are underway to understand the mechanisms related to azole resistance in these isolates.

Another noteworthy finding of our study was the emergence of echinocandin resistance in the Southeast Asia region. In the post-echinocandin era, there have been increasing reports of echinocandin treatment failures in most clinically-relevant species, especially in *C. glabrata* [7, 21–24]. Fortunately, resistance rates remained rare in the local context. There were only three (1.1%) isolates which were echinocandin-resistant, of which two had fks mutations. To the best of our knowledge, this is the first incidence of fks mutations in *Candida* bloodstream isolates other than *C. glabrata* identified locally. While the fks mutations identified in our isolates have been previously described, it is interesting to note that resistance developed rapidly (within 4 days of exposure to caspofungin) in one of the patients. Development in resistance has been primarily related to prolonged use of echinocandins, which was observed in the other patient, who had received 30 days of caspofungin prior to *Candida* isolation [22].

Our study observed a high 30-day mortality rate of 49%. Like many previous studies, we found that mortality was associated with severity of illness at onset of candidemia, suggesting that the poor outcomes of patients with candidemia is likely related to the poor prognosis of these patients with multiple comorbidities [25]. Receipt of renal replacement therapy was also associated with 30-day mortality. This could be an indication of the underlying organ dysfunction contributing to severity of illness. Drains placement prior to *Candida* isolation was found to be protective, suggesting that perhaps source control could contribute to better survival in patients with secondary candidemia.

Initial antifungal choice did not appear to be associated with mortality in our study. Although the Infectious Diseases Society of America guidelines have recommended the use of an echinocandin as a first-line agent, randomised controlled trials conducted so far have yet to conclusively demonstrate superiority of one agent over another [26–28]. A recent study has also illustrated that clinical severity, rather than initial antifungal strategy, was significantly correlated with mortality [25]. One reason why we were unable to detect any association of initial antifungal choice with mortality could be because we did not account for the appropriateness of the therapy in terms of dosing. Furthermore, pharmacokinetic variability can result in fluctuating antifungal levels in individual patients [29]. Perhaps, the impact of initial antifungal choice on treatment outcomes can be better elucidated if antifungal dosing was individualised, such as through the use of therapeutic drug monitoring. This therapeutic approach is currently being explored in our institution.

Although a large number of our patients received antifungals in a timely fashion, there was still a delay in therapy for some patients, with some receiving antifungals more than a week after cultures were taken. The time to administration of antifungals could be limited by the lack of rapid diagnostic tests available in our institution. It takes an average of two days to report a positive *Candida* blood culture, and in some instances even up to a week.

Our study was not without limitations. This was a single-centre study and our results might not be extrapolated to other institutions as the epidemiology of
candidemia can be highly institution-specific. The retrospective nature of the study also precluded the analysis of impact of time of catheter removal on mortality. Nevertheless, this study provides important epidemiological findings which are instrumental in designing strategies for better management of candidemia in our institution.

Conclusions
While incidence of candidemia appeared to be stable, incidence of C. glabrata is increasing. C. glabrata and C. tropicalis contributed to majority of the candidemia cases in our institution. Decreasing azole susceptibilities to C. tropicalis and the emergence of echinocandin resistance suggests that susceptibility patterns may no longer be sufficiently predicted by speciation in our institution. Routine antifungal susceptibility, particularly for C. tropicalis, might be essential to guide clinician to effectively manage patients with invasive Candida infections. Candidemia was associated with high mortality, and antifungal stewardship efforts in individualising antifungal dosing through therapeutic drug monitoring should be further explored to improve outcomes in this population.

Abbreviations
ATCC: American Type Culture Collection; CI: Confidence interval; CLSI: Clinical and Laboratory Standards Institute; ECV: Epidemiological cut-off values; ICU: Intensive care unit; MIC: Minimum inhibitory concentration; OR: Odds ratio; PCR: Polymerase chain reaction; SAPS: Simplified acute physiology score; SGH: Singapore General Hospital

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Availability of data and materials
Please contact author for data requests.

Authors’ contributions
JQT, SRC, SJL, SYC, HL, TPL and ALT participated in the microbiological and/or molecular experiments. JQT, SRC, SJL, SYC, HL, TPL and ALT participated in the microbiological and/or molecular experiments. JQT, SRC, SJL, SYC, HL, TPL and ALT participated in the microbiological and/or molecular experiments. JQT, SRC, SJL, SYC, HL, TPL and ALT participated in the microbiological and/or molecular experiments. JQT, SRC, SJL, SYC, HL, TPL and ALT participated in the microbiological and/or molecular experiments.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The research protocol was approved by the Singhealth Centralised Institutional Review Board (2013/987/D). Informed consent was waived in view of retrospective nature of study.

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References
1. Magill SS, Edwards JR, Bantberg W, Beldavs ZG, Dumyatgi G, Kainer MA, Lynfield R, Maloney M, McAllister-Holod F, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK. Emerging Infections Program Healthcare-Associated I. Antimicrobial Use Prevalence Survey T. Multistate point-prevalence survey of health care-associated infections. N Engl J Med. 2014;370(13):1198–208.
2. Basseti M, Merelli M, Ansaldi F, de Florentis D, Santar A, Scarpato C, Gallegari A, Righi E. Clinical and therapeutic aspects of candidemia: a five year single centre study. PLoS One. 2015;10(5):e0127534.
3. Pfaffer M, Neofytos D, Dekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance9) registry, 2004–2008. Diagn Microbiol Infect Dis. 2012;74(4):323–31.
4. Dekema D, Arbeelv Sil, Boyken L, KroesENDER, Pfaffer M. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis. 2012;73(1):4–9.
5. Hassan I, Powell G, Sidhu M, Hart WM, Denning DW. Excess mortality, length of stay and cost attributable to candidemia. J Infect. 200095(5):360–5.
6. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur J Clin Microbiol Infect Dis. 2006;25(7):419–25.
7. Alexander BD, Johnson MD, Pfeiffer CD, Jimenez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlinc DS, Pfaffer MA. Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis. 2013;56(12):1724–32.
8. Montagna MT, Lorero G, Borghi E, Amato G, Andreoni S, Campion L, Lo Cacio G, Lombardi G, Luzzaro F, Manso E, Müssap M, Péceli P, Perlin S, Tangorra E, Tronci M, Iatta R, Morace G. Candidemia in intensive care unit: a nationwide prospective observational study (GISA-3 study) and review of the European literature from 2000 through 2013. Eur Rev Med Pharmacol Sci. 2014;18(S):661–74.
9. Guinea J. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect. 2014;20 Suppl 6:5–10.
10. Tan BH, Chakrabarti A, Li RY, Patel AK, Watchanaranon SP, Liu Z, Chindamporn A, Tan AL, Sun PL, Wu UI, Chen YC. Asia Fungal Working Group. Incidence and species distribution of candidaemia in Asia: a laboratory-based surveillance study. Clin Microbiol Infect. 2015;21(10):946–53.
11. Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakuikkeeree M, Choudhury S, Chen YH, Shin JH, Kraitisin P, Mendoza M, Prabhu K, Supparapinayo K, Tan AL, Phan XT, Tran TT, Nguyen GB, Doan MP, Huyhn VA, Nguyen SM, Tran TB, Van Pham H. Antifungal susceptibility of invasive Candida bloodstream isolates from the Asia-Pacific region. Med Mycol. 2016;54(5):471–7.
12. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Fourth Informational Supplement. CLSI document M27-S4. Wayne: Clinical and Laboratory Standards Institute; 2012.
13. Canton E, Pernan J, Hervas D, Iniguez C, Navarro D, Echeverria J, Martinez-Alarcon J, Fontanil D, Gornal-Sard B, Buenda B, Torroba L, Ayats J, Brutus A, Sanchez-Reu F, Fernandez-Natal I. Comparison of three statistical methods for establishing tentative wild-type population and epidemiological cutoff values for echinocandins, amphotericin B, fluconazole, and six Candida species as determined by the colormetric Sensititre YeastOne method. J Clin Microbiol. 2012;50(2):3921–6.
14. Canton E, Pernan J, Iniguez C, Hervas D, Lopez-Hontangas JL, Pina-Vaz C, Camarena JJ, Campos-Herrero I, Garcia-Garcia I, Garcia-Tapia AM, Guna R, Merino P, Perez del Molino L, Rubio C, Suarez A, Group FS. Epidemiological
cutoff values for fluconazole,itraconazole, posaconazole, and voriconazole for six Candida species as determined by the colorimetric Sensititre YeastOne method. J Clin Microbiol. 2013;51(8):2691–5.

15. Espinel-Ingroff A, Alvarez-Fernandez M, Canton E, Carver PL, Chen SC, Teo et al. Antimicrobial Resistance and Infection Control 29. Lewis RE. Current concepts in antifungal pharmacology. Mayo Clin Proc. 2011;86(8):805–17.