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Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility

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Abstract

This study reports for the first time, to our knowledge, descriptive epidemiological data for 18 invasive *Candida* isolates from Pakistan, including species identification and antifungal susceptibility against fluconazole, itraconazole, voriconazole, caspofungin, micafungin, anidulafungin and amphotericin. Risk factors for invasive candidiasis (IC) were determined for 96 patients from Karachi, Pakistan. In adults and neonates, *Candida tropicalis* (38 and 36 %, respectively) was the most common species, followed in adults by *Candida parapsilosis* (17.8 %), *Candida glabrata* (15.9 %) and *Candida albicans* (12.3 %). *C. albicans* (21 %) was the second most common in neonates. In children, *C. albicans* (31.9 %), *C. tropicalis* (26.4 %) and *C. parapsilosis* (19.4 %) were the most common. *C. albicans* IC was significantly associated with paediatric age [crude odds ratio (COR) 3.46, 95% confidence interval (CI) 1.63–7.32]. Rare species made up 17.5% of the total isolates studied. Resistance to fluconazole was seen in *C. glabrata* (15.0%) and *Candida krusei* (100.0%). Only one isolate (*C. glabrata*) was resistant to all three echinocandins. Low MICs of fluconazole for 98% (184/188) of isolates tested support its continued use as an empiric therapy for IC. Non-*C. albicans* IC was associated with the use of β lactam inhibitor combinations (COR 3.16, 95% CI 1.05–9.57). Use of healthcare devices was documented in 85.4% of IC patients, whilst 75.0% had been admitted to special care units. Surprisingly, 66.7% of patients with IC were not obviously immunosuppressed. The high frequency of modifiable risk factors in this population indicates that candidaemia can be reduced with stringent antibiotic and infection control measures. These data will be useful for empiric selection of antifungals in Karachi, and contribute to global assessments of antifungal resistance.

INTRODUCTION

Invasive candidiasis (IC) is a severe illness with a high mortality and increasing overall cost of management (Colombo *et al*., 2006; Hassan *et al*., 2009; Horn *et al*., 2009; Pfaller *et al*., 2011d). Advances in medical technology and therapeutics have resulted in increasing numbers of invasive fungal infections including IC (Diaz & Fell, 2004). Individuals considered at greatest risk of IC are premature infants, patients receiving broad-spectrum

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antibiotics or anti-cancer chemotherapy (Picazo *et al*., 2008) and those with immunodeficiencies such as haematopoietic stem-cell transplant or solid-organ transplant recipients, profound neutropenia or diabetes (Kontoyiannis *et al*., 2010; Pappas *et al*., 2010).

Globally, *Candida albicans* is the most frequently reported species; however, the emergence of non-*C. albicans Candida* species with resistance to fluconazole is of concern, highlighted by recent surveillance data (Pfaller *et al*., 2011d). As inappropriate initial therapy for IC can result in an adverse outcome, accurate identification of the species responsible for IC and knowledge of their corresponding susceptibility patterns are essential. It is also important to recognize populations at greater risk of IC in the healthcare setting. In the absence of a national surveillance system for IC in Pakistan, laboratory data are epidemiologically useful to identify susceptible patient populations, monitor trends and detect emerging resistance. Most of the clinical laboratories in Pakistan rely on phenotypic identification methods to report *Candida* species; however, current genotypic methods of identification are considered more reliable, especially for the less common species (Deak *et al*., 2010; Wang *et al*., 2008).

In this study, we determined the spectrum of invasive *Candida* isolates in samples collected from major cities of Pakistan, confirmed their identification to the species level with molecular methods and determined their susceptibility patterns. We also analysed risk factors associated with IC and have provided descriptive epidemiological information on these patients.

METHODS

Study background

The study was conducted in the clinical laboratory of the Aga Khan University Hospital (AKUH), Karachi, Pakistan. The laboratory has a national specimen collection network with more than 175 collection points in major cities and towns across the country. Samples are processed in the clinical laboratory based in Karachi. The laboratory data were collected from nine major cities in Pakistan, the majority from Karachi.

Definitions

IC was defined as the isolation of *Candida* species from normally sterile specimens, such as blood, cerebrospinal fluid, peritoneal fluid, pleural fluid, synovial fluid and bile, and from the tips of central venous catheters and from ventriculoperitoneal shunts. Patients were considered children if they were ≤14 years, and neonates if they were <30 days. Isolation of the same *Candida* species within 15 days was considered a single episode of IC. Sepsis was defined as presence of hypotension (systolic blood pressure <90 mm Hg or the need for inotropes), temperature <36 or >38 °C and a white blood cell count of <4000 or >10 000 mm^{-3}. Admission to adult, paediatric or neonatal intensive care units was considered ‘special care stay’. Nosocomial infections were those acquired after >48 h of admission to, or within 90 days of discharge from, a healthcare facility. A central line-associated bloodstream infection (CLABSI) was considered if there was no other source of bloodstream infection except a central venous line. A β-lactam inhibitor combination (BLIC) was cephalosporin or penicillins combined with tazobactam, clavulanic acid or sulbactam.

Patients on dialysis received either peritoneal or haemodialysis. Neutrophilia was defined as >70 % neutrophils on a peripheral blood smear, whilst neutropenia was defined as an absolute neutrophil count of <1000 mm^{-3}. 'Prior antifungal use' was the administration of fluconazole, voriconazole, itraconazole or amphotericin B at least 24 h before collection of the specimen yielding *Candida* species. Healthcare devices included central venous lines [femoral, jugular or subclavian central venous catheters, haemodialysis catheters, peripherally inserted central catheter lines, portacath or Hickman lines], permanent or transient pacemakers, ventilators, abdominal or chest drains, peritoneal dialysis catheters, percutaneous nephrostomy tubes, suprapubic catheters, percutaneous endoscopic gastrostomy tubes, pancreatic duct stents, endoventricular drains, ventriculoperitoneal shunts and orthopaedic implants. 'Complicated intra-abdominal infection' was defined as abdominal infection spreading beyond the hollow viscus into the peritoneal cavity, resulting in peritonitis or intra-abdominal abscess. Community-acquired or healthcare-associated pneumonia or urinary tract infections were all included in 'pneumonia' or 'urinary tract infection', respectively. Total parenteral nutrition was the administration of amino acids and lipids via the intravenous route when enteral feeding was not possible or inadequate. 'Immunosuppressed status' was when the patient was receiving steroids, cyclosporine, azathioprine or anti-cancer chemotherapy, or was neutropenic, or was a cancer or transplant patient.

Risk factor study

Medical records of 96/183 patients (118/207 isolates) registered with AKUH were retrieved for a retrospective clinical data review. Risk factors, underlying disease and the course of clinical illness were recorded.

Yeast identification

During the study period (January 2006 to May 2009), 207 sterile specimens from 180 patients yielded *Candida* species. After excluding duplicates from the same episode of candidiasis, 188 isolates were included. Five patients had candidaemia with multiple species, whilst two had recurrence of IC with the same species. The specimens were 166 blood cultures, 15 sterile fluids (seven peritoneal, three pleural, three cerebrospinal fluid and two others), six central catheter tips and one ventricular shunt end tip. The geographical origin of these isolates was as follows: 134 were from AKUH, 34 were from other healthcare facilities in Karachi and 20 were from additional cities in Pakistan (seven from Lahore, four from Multan, three from Hyderabad, two from Quetta and one each from Peshawar, Rawalpindi, Bahawalpur and Rahimyar Khan). Species identification was based on conventional phenotypic characteristics: production of a germ tube, morphology on BBL BiGGY Agar (BD), growth with cycloheximide, urease production, morphology on cornmeal/Tween 80 agar and the identification profile generated using API 20C AUX (bioMe´rieux). Isolates were stored in glycerol phosphate buffer at −80 °C. Their identification was also confirmed using either a Luminex multianalyte profiling assay with the ITS2 target or DNA sequencing as described previously by Das *et al*. (2006) and Deak *et al*. (2010). Agreement between phenotypic and molecular identification was >90 % for common species such as *C. albicans, Candida tropicalis and Candida parapsilosis*.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by broth microdilution with fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin and micafungin as described by the Clinical and Laboratory Standards Institute (CLSI, 2008) using frozen RPMI microbroth trays custom manufactured by TREK Diagnostics. Results were read visually after 24 h of incubation as the lowest concentration of drug that caused a significant decrease in growth compared with the control well. Recently approved although not yet published CLSI 24 h resistance breakpoints for fluconazole, voriconazole and the echinocandins were used (CLSI, 2008; Pfaller *et al*., 2010a, 2011a, c). *C. albicans, C. tropicalis* and *C. parapsilosis* with MICs ≥8 μg ml^{-1} and *C. glabrata* with an MIC ≥64 μg ml^{-1} were considered resistant to fluconazole, whilst *Candida krusei* was considered intrinsically resistant to fluconazole. *C. albicans, C. tropicalis* and *C. parapsilosis* with MICs ≥1 mg ml^{21} were considered resistant to voriconazole. *C. albicans, C. tropicalis* and *C. krusei* with MICs ≥1 μg ml^{-1} were considered resistant to caspofungin, micafungin and anidulafungin; *C. para-psilosis* with MICs ≥8 μg ml^{-1} was considered resistant to caspofungin, micafungin and anidulafungin; *Candida glabrata* with MICs ≥0.5 μg ml^{-1} was considered resistant to caspofungin and anidulafungin and with an MIC ≥0.25 μg ml^{-1} was resistant to micafungin. In the absence of clinical breakpoints, epidemiological cut-off values (ECVs) were used to interpret MICs as those conforming to wild-type or non-wild-type for a particular antifungal agent against a specific species. ECVs for fluconazole were 8 μg ml^{-1} for *Candida guilliermondii*, 4 μg ml^{-1} for *Candida pelliculosa*, 2 μg ml^{-1} for *Candida lusitaniae* and *Candida orthopsilosis* and 1 μg ml^{-1} for *Candida kefyr*. ECVs for voriconazole were 0.25 μg ml^{-1} for *C. guilliermondii* and *C. pelliculosa*, 0.03 μg ml^{-1} for *C. lusitaniae*, 0.06 μg ml^{-1} *C. orthopsilosis* and 0.015 μg ml^{-1} for *C. kefyr* (Pfaller *et al*., 2011b). Similarly, ECVs of anidulafungin were 4 μg ml^{-1} for *C. guilliermondii*, 2 μg ml^{-1} for *C. lusitaniae* and *C. orthopsilosis*, and 0.25 μg ml^{-1} for *C. kefyr*, and those for caspofungin were 2 μg ml^{-1} for *C. guilliermondii*, 1 μg ml^{-1} for *C. lusitaniae*, 0.5 μg ml^{-1} for *C. orthopsilosis*, 0.12 μg ml^{-1} for *C. pelliculosa* and 0.03 μg ml^{-1} for *C. kefyr* (Pfaller *et al*., 2011b). ECVs for micafungin were 2 μg ml^{-1} for *C. guilliermondii*, 1 μg ml^{-1} for *C. orthopsilosis*, 0.5 μg ml^{-1} for *C. lusitaniae* and 0.12 μg ml^{-1} for *C. kefyr* (Pfaller *et al*., 2011b). Amphotericin B MICs were obtained by Etest on RPMI agar by making a lawn with a 0.5 McFarland standard inoculum and incubating it for 24 h at 35 °C. The MICs of amphotericin and itraconazole were also interpreted according to ECVs (Pfaller *et al*., 2012). For amphotericin, the ECV for *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. guilliermondii* and *C. lusitaniae* was 2 μg ml^{-1}. For itraconazole, ECVs were 0.12 μg ml^{-1} for *C. albicans*, 0.5 μg ml^{-1} for *C. parapsilosis, C. tropicalis* and *C. lusitaniae*, 1 μg ml^{-1} for *C. krusei* and *C. guilliermondii* and 2 μg ml^{-1} for *C. glabrata*.

Statistical analysis

Data were entered in Microsoft Access (2007) and transferred to SPSS version 19.0 for analysis. The frequencies of *Candida* species and their susceptibility profile and risk factors were determined. Patient characteristics associated with *Candida* species were evaluated using a χ^2 test. The crude odds ratio (COR) and 95 % confidence interval (CI) were calculated for variables found to have significant associations.

RESULTS

Demographics, clinical characteristics and risk factors

A cross-sectional *in vitro* observational study was performed to determine the species spectrum in IC, together with the susceptibility profiles of the isolates and the risk factors associated with the disease. The demographics of 180 patients revealed a male/female ratio of 1.47. The mean age of 67 paediatric patients was 1.9 years (0 days–14 years), with 33 neonates. The mean age of 113 adults was 47.4 years (15–96 years), and 83 (73.5 %) of these were <65 years of age. The crude mortality rate was 52.1 %.

Further clinical information was available on 96 patients seen at AKUH (Tables 1 and 2). In this group, *C. albicans* IC was significantly associated with children and neonates (COR 3.46, 95% CI 1.63–7.32) and not with adults (COR 0.29, CI 0.14–0.61), whilst *C. glabrata* was associated with adults (COR 6.44, 95 % CI 1.45–28.69) and not with the paediatric group (COR 0.16, 95 % CI 0.04–0.69). However, the number of neonates with available clinical information was too small to compare separately for the presence of other risk factors.

Among these patients, overall use of carbapenems and BLICs was documented in 53 and 46% of cases, respectively. Paediatric IC, including neonates, was significantly associated with increased use of cephalosporins (COR 4.14, 95 % CI 1.52–11.26) and decreased use of BLICs (COR 0.13, 95% CI 0.35–0.47) and vancomycin (COR 0.33, 95 % CI 0.11–0.98) compared with adults.

In 91.6 % of cases, IC was acquired nosocomially. Use of healthcare devices of central venous catheters, ventilators and abdominal and pleural cavity drains was documented in 85.4 % of patients with IC, whilst 75.0 % of these individuals had spent time in special/intensive care units. Administration of total parenteral nutrition was also found to be significantly higher among children and neonates (COR 3.58, 95 % CI 1.25–10.23). Using the study criteria, 66.7 % of the study population were not overtly immunosuppressed.

Table 2 shows the association of risk factors with isolation of the four most common *Candida* species. IC with *C. tropicalis* was significantly associated with the presence of chronic liver disease (COR 5.16, 95 % CI 1.19–22.33), sepsis (COR 3.71, 95 % CI 1.16–11.92) and the need for dialysis (COR 4.21, 95 % CI 1.42–12.55). Candidaemia with *C. albicans* was seen less frequently in cases where there was use of BLICs (COR 0.32, 95 % CI 0.10–0.96). Isolation of *C. parapsilosis* was higher with established CLABSI (COR 4.53, 95% CI 1.35–15.25) and *C. glabrata* from diabetic patients (COR 5.08, 95 % CI 1.21–21.34). The risk of isolation of *C. glabrata* was found to be low with the use of carbapenem antibiotics (COR 0.18, 95 % CI 0.03–0.95).

Species distribution

The most commonly isolated *Candida* species overall was *C. tropicalis* (32.5 %), followed by *C. albicans* (20.2 %) and *C. parapsilosis* (15.0 %). Rare species including *C. guilliermondii*, *C. metapsilosis*, *C. orthopsilosis*, *C. viswanathii*, *C. pelliculosa*, *C. utilis*, *C.*

fabianii, *C. rugosa*, *C. kefyr* and two novel *Candida* species comprised 17.5 % of total IC isolates (Tables 3 and 4).

Adults, neonates and children displayed different species distribution profiles (Fig. 1). In adults and neonates, *C. tropicalis* (38 and 36 %, respectively) was common, whilst in children the predominant isolate was *C. albicans* (44 %). The isolation rates of *C. glabrata* were minimal (6 %) in neonates and 0 % in children.

Antifungal susceptibility

Table 3 shows the MIC_{50} and MIC_{90} values, the MIC range and the percentage of resistant isolates. In general, most isolates were susceptible to the drugs tested. Resistance to fluconazole was seen in *C. glabrata* (15 %) and *C. krusei* (100 %). For the rare *Candida* species, the MIC_{90} values of fluconazole were ≤4 μg ml^{-1}; however, one *C. pelliculosa* strain showed non-wild-type behaviour in respect to fluconazole with an MIC of 8 μg ml^{-1} (Tables 3 and 4). Non-wild-type behaviour in regard to itraconazole was seen among *C. albicans* (23.7 %), *C. tropicalis* (19.7 %) and *C. glabrata* (10.0 %) isolates (Table 3). Only one *C. glabrata* isolate was resistant to voriconazole. All isolates displayed amphotericin B MICs of <1.0 μg ml^{-1}, well below the ECV of 2.0 μg ml^{-1}. All isolates except one *C. glabrata* strain (caspofungin MIC of 0.5 μg ml^{-1}, anidulafungin MIC of 1.0 μg ml^{-1} and micafungin MIC of 0.5 μg ml^{-1}) were susceptible to echinocandins, as shown in Table 3.

DISCUSSION

This assessment of the epidemiology of IC in Pakistan showed *C. tropicalis* to be the species isolated most frequently from adult and neonatal IC patients (33 % of cases). This finding contrasts with reports from the USA, Europe, South America and the Far East, which have reported *C. albicans* as the most common species (Colombo *et al*., 2006; Falagas *et al*., 2010; Odds *et al*., 2007), with the most frequent non-*C. albicans* species being either *C. parapsilosis* in Southern Europe and South America or *C. glabrata* in the USA and UK. We found *C. albicans* to be the fourth most common *Candida* species among adult IC pathogens in Pakistan after *C. tropicalis*, *C parapsilosis* and *C. glabrata*. Our results support regional data from India showing *C. tropicalis* to be the most prevalent species in hospital-based surveys (Falagas *et al*., 2010; Kothari & Sagar, 2009). Studies from Singapore, Taiwan and Brazil have also reported *C. tropicalis* as the most common non-*C. albicans* species (Liu *et al*., 2010; Pereira *et al*., 2010; Tan *et al*., 2010). *C. tropicalis* has been reported as an emerging pathogen in hospital-based surveys of IC in Singapore and Hong Kong (Chai *et al*., 2007; Yap *et al*., 2009). Compared with data from the ARTEMIS disc diffusion surveillance, our isolation rates of *C. tropicalis* and *C. guilliermondii* were much higher than those reported from Africa, the Middle East and Asia Pacific (Pfaller *et al*., 2010a). The emergence of *C. tropicalis* causing IC in Indian studies has been attributed to increased fluconazole resistance and virulence, as portrayed by an increased rate of growth and the production of proteinases facilitating invasion (Basu *et al*., 2011; Kothavade *et al*., 2010). We did not find overt fluconazole resistance in Pakistani isolates of *C. tropicalis*, but the pathogenicity of this species requires further study. An association of *C. tropicalis* fungaemia with severity indicators such as sepsis (COR 3.71, 95 % CI 1.16–11.92; Table 2)

and the need for dialysis (COR 4.21, 95 % CI 1.42–12.55; Table 2) supports our theory of increasing virulence in this species. It was also found more frequently in patients suffering from chronic liver disease (COR 5.16, 95 % CI 1.19–22.33), a result not particularly found in other studies but which possibly reflects increased *C. tropicalis* gastrointestinal carriage, leading to candidaemia in such patients (Kusne *et al*., 1994; Nucci & Colombo, 2007).

Our data showed a clear distinction between the spectrum of adult, neonatal and paediatric (age >1 month) IC pathogens, with *C. albicans*, *C. tropicalis* and *C. parapsilosis* being the three main paediatric IC agents. The age distribution of species in an international SENTRY survey for antifungal resistance showed *C. albicans* as the most common species isolated, irrespective of age group (Pfaller *et al*., 2010b). Conversely, our data showed a clear predominance of *C. albicans* (44 %) only in children, whilst in adults and neonates ~70 % of IC was caused by species other than *C. albicans*. This species distribution among the paediatric age group is supported by other reports published on the epidemiology of candidiasis in children (Ariff *et al*., 2011; Zaoutis, 2010). Differences in the epidemiology of IC in adults and children suggest that pressures, such as the spectrum of antibiotics used or gut colonization in Asian individuals, influencing the selection of non-*C. albicans* species in adults are not found as frequently in children. Although the distribution of species among neonates and adults appeared similar compared to that among children >1 month of age, a lack of clinical information unfortunately limited our ability to assess statistically the risk factors for neonates separately. Use of vancomycin and BLICs, an established risk factor for non-*C. albicans* species (Lin *et al*., 2005) and also significantly associated with non-*C. albicans* IC in this study (COR 0.32, 95 % CI 0.10–0.96; Table 2), was found to be significantly low at 22.7 and 13.6% in children and neonates, respectively (COR 0.33, 95 % CI 0.11–0.98 and COR 0.13, 95 % CI 0.35–0.47, respectively; Table 1).

The study of risk factors in our patients suggested that IC was mainly a nosocomial infection (91.6 % of cases), with most patients requiring intensive care and broad-spectrum parenteral antibiotic therapy. Although AKUH is a tertiary care hospital and a bone-marrow transplant centre, more than two thirds of the patients with IC in AKUH were without obvious immunocompromised status: only 9.4 % were neutropenic. Most studies of risk factors associated with IC are either hospital-based, intensive care unit or specific unit surveys (e.g. burns unit, transplant registry, etc.). A study from Greece that compared risk factors in immunocompetent and immunocompromised hosts (Dimopoulos *et al*., 2007) determined the risk factors to be the use of central venous catheters and urinary catheters, prolonged hospital stay and intra-abdominal infections in immunocompetent hosts and haematopoietic stem-cell transplant recipients, and neutropenia in immunocompromised patients. In hospital-based surveys, stay in special care units and infections with other nosocomial organisms were associated with high mortality, whilst central intravascular catheters and previous azole therapy were significantly associated with *C. parapsilosis* and *C. krusei*, respectively (Dimopoulos *et al*., 2007; Erdem *et al*., 2010; Ortega *et al*., 2011). Our study also found *C. parapsilosis* to be significantly associated with CLABSI (COR 4.53, 95 % CI 1.35–15.25; Table 2). Recently, the Infectious Disease Society of America has published guidelines for the prevention of intravascular catheter-related bloodstream infections, emphasizing barrier and aseptic precautions, clean sites of insertion and timely removal of

central lines (O'Grady *et al*., 2011). In our patients, the high rates of modifiable risk factors, such as the use of central lines, staying in a special care unit and the use of broad-spectrum antibiotics, suggest that a significant proportion of IC may be preventable. Thus, adhering to the central line bundle and good antibiotic monitoring may effectively reduce IC rates, highlighting the importance of infection control.

The association of *C. glabrata* with diabetes is well established (Bader *et al*., 2004); however, the association of carbapenem use with a decreased risk of *C. glabrata* infection needs further investigation, as this may be due to the small sample size.

C. tropicalis, C. albicans, C. parapsilosis, C. glabrata and C. krusei comprised only 83 % of this collection, although they generally comprise >90 % of most surveillance collections (Pfaller & Diekema, 2007). Higher rates of recovery of uncommon species such as *C. guilliermondii, C. lusitaniae* and other rare species (17.5 % of the total IC isolates) in this study suggest that investment in molecular yeast identification methodology would be worthwhile. However, the phenotypic identification methods employed had good species agreement rates with molecular identification, with the exception of *C. guilliermondii* and rare *Candida* species, and may be used until molecular identification becomes more available in this country.

Resistance to azole antifungals, particularly fluconazole, in certain species has been increasing in recent years in the USA and Europe. Studies from Europe, South America and the USA reported low resistance rates to fluconazole and itraconazole before 2005 (Pfaller & Diekema, 2007), but data from the latter half of the decade show emerging resistance not only against azoles but also against echinocandins in nosocomial isolates (Pfaller et al., 2011d). Our isolates did not manifest high rates of fluconazole resistance except among *C. glabrata* and *C. krusei* isolates.

All *Candida* species in our study had low amphotericin B MICs, reinforcing the reliability of amphotericin B as an empirical choice. Although all our isolates were echinocandin naïve, as caspofungin has only recently become available in Pakistan, one *C. glabrata* isolate showed resistance to echinocandins, suggesting that selection pressures may play a role in the future as echinocandin use increases. Regardless of this, where cost is not an issue and *C. glabrata* and *C. krusei* have been isolated, echinocandins can be considered good choices for directed antifungal therapy in Pakistan. There are, however, reports from other parts of the world of resistance among nosocomial C. glabrata isolates against echinocandins (Pfaller *et al*., 2011d; Zimbeck *et al*., 2010).

With the infrequent isolation of *C. glabrata* and *C. krusei* and fluconazole resistance rates of 0 % among other *Candida* species in this study, fluconazole may be considered a good empiric choice. Good antibiotic monitoring could save the use of echinocandins for confirmed cases of *C. glabrata* and *C. krusei*, keeping the cost of therapy and antifungal exposure minimal, as the costs attributable to candidaemia are already high (Hassan *et al*., 2009). As the clinical use of antifungals increases we may see the emergence of resistance, and accurate identification to the species level as well as routine antifungal susceptibility testing are likely to have greater implications. There is evidence of changes in epidemiology

and increasing rates of azole resistance in developed countries, which advocates continuing surveillance for species and the antifungal susceptibility spectrum (Falagas *et al*., 2010; Pfaller *et al*., 2011d).

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Abbreviations

AKUH	Aga Khan University Hospital
BLIC	β-lactam inhibitor combination
CI	confidence interval
CLABSI	central line-associated bloodstream infection
COR	crude odds ratio
ECV	epidemiological cut-off value
IC	invasive candidiasis

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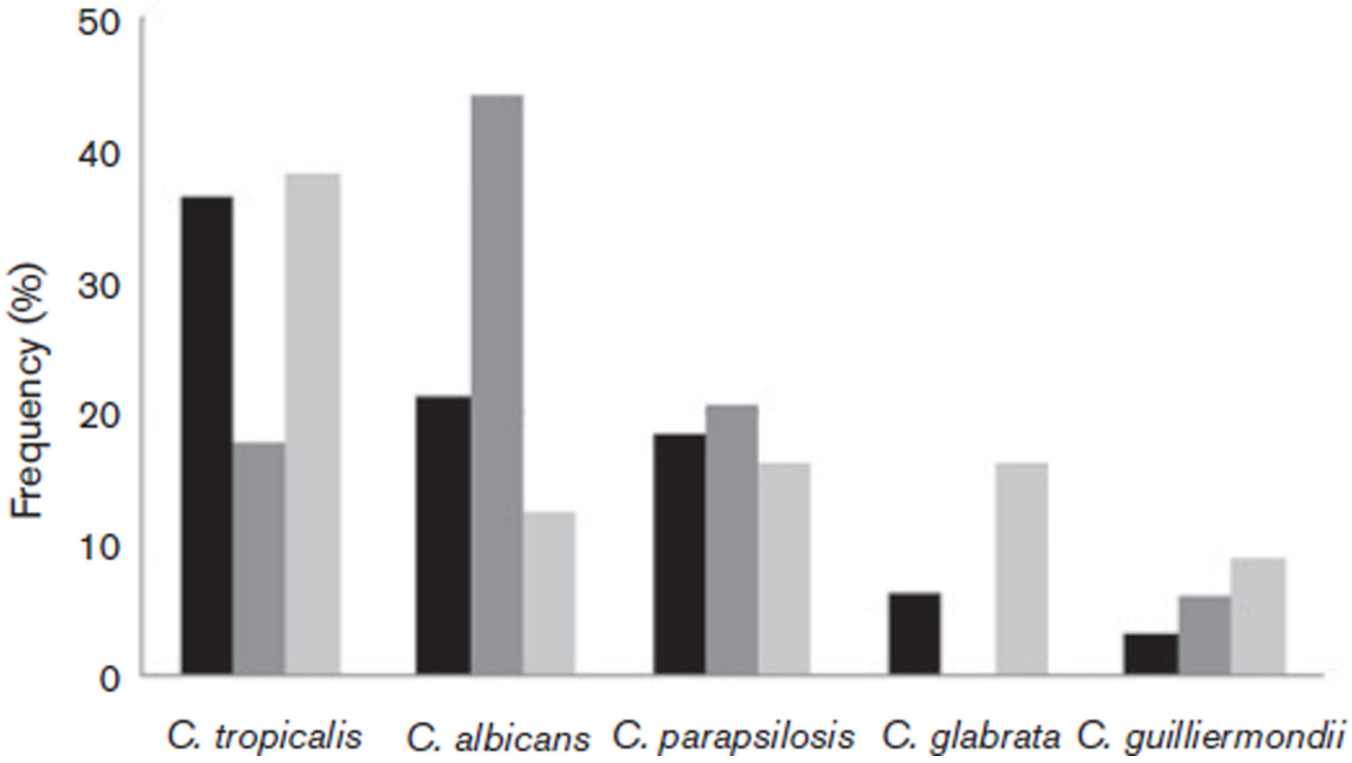

Fig. 1.

Distribution of species based on the age of IC patients in this study. Black bars, neonates; dark grey bars, children; light grey bars, adults.

Table 1

Demographics and clinical characteristics of patients with IC. Statistically significant differences (*P*,0.05) within age groups are shown in bold. COR and 95 % CI have been shown for significant associations only. –, Difference not statistically significant; NA, not applicable.

Variable	All cases (%)	Children	Adults	*P* value	COR (95% CI)
Mean age in years (range)	30.48 (0–96)	NA	NA	NA	NA
No. of males (%)	107 (59.4)	NA	NA	NA	NA
No. of children (%)	67 (37.2)	NA	NA	NA	NA
Presence of underlying risk factors in 96 medical records reviewed		NA	NA	NA	NA
Any malignancy	21 (21.9)	4 (18.2)	17 (23.0)	–	–
Haematological malignancy	6 (6.3)	2 (9.1)	4 (5.4)	–	–
CLABSI	24 (25.0)	5 (22.7)	19 (25.7)	–	–
Chronic liver disease	9 (9.4)	0 (0.0)	9 (12.2)	–	–
Complicated intra-abdominal infection	21 (21.9)	2 (9.1)	19 (25.7)	–	–
Central venous catheters	64 (66.7)	14 (63.6)	50 (67.6)	–	–
Diabetes	19 (19.8)	0 (0.0)	19 (25.7)	–	–
Dialysis	17 (17.7)	2 (9.1)	15 (20.3)	–	–
Immunosuppressed status	32 (33.3)	6 (27.3)	26 (35.1)	–	–
Neutropenia	9 (9.4)	3 (13.6)	6 (8.1)	–	–
Nosocomial IC	88 (91.6)	19 (67.9)	69 (84.1)	–	–
Pneumonia	28 (29.2)	6 (27.3)	22 (29.8)	–	–
Prior antifungal use	19 (19.8)	3 (13.6)	16 (21.6)	–	–
Sepsis	68 (70.8)	15 (68.2)	53 (71.6)	–	–
Stay in special care units	72 (75.0)	14 (63.6)	58 (78.4)	–	–
Total parenteral nutrition use	21 (21.9)	9 (40.9)	12 (16.2)	**0.040**	3.58 (1.25–10.23)
Transplant	2 (2.1)	0 (0.0)	2 (2.7)	–	–
Use of BLIC	44 (45.8)	3 (13.6)	41 (55.4)	**0.001**	0.13 (0.35–0.47)
Use of carbapenems	51 (53.1)	15 (68.2)	36 (48.6)	–	–
Use of cephalosporins	36 (37.5)	14 (63.6)	22 (29.8)	**0.004**	4.14 (1.52–11.26)
Use of healthcare devices	82 (85.4)	16 (72.7)	66 (89.2)	–	–
Use of vancomycin	40 (41.7)	5 (22.7)	35 (47.3)	**0.040**	0.33 (0.11–0.98)
Urinary tract infection	19 (19.8)	2 (9.1)	17 (23.0)	–	–

Variable	All cases (%)	Children	Adults	*P* value	COR (95% CI)
Crude mortality	50 (52.1)	10 (45.5)	40 (54.1)	–	–

Table 2

Association of risk factors with the most prevalent *Candida* species A *P* value of <0.05 is considered statistically significant.

Species	Risk factor	*n*/Total*	Frequency (%)	*P* value	COR (95% CI)
C. tropicalis (*n*=30)	Chronic liver disease	6/9	66.7	0.017	5.16 (1.19–22.33)
	Dialysis	10/17	58.8	0.007	4.21 (1.42–12.55)
	Sepsis	26/68	38.2	0.021	3.71 (1.16–11.92)
C. albicans (*n*=20)	Paediatric age	23/70	32.9	0.001	3.46 (1.64–7.32)
	Adult age	14/113	12.4	0.001	0.29 (0.14–0.61)
	Use of BLIC	5/44	11.4	0.036	0.32 (0.10–0.96)
C. parapsilosis (*n*=13)	CLABSI	7/24	29.2	0.010	4.53 (1.35–15.25)
C. glabrata (*n*=11)	Adult age	18/113	15.9	0.015	6.44 (1.45–28.69)
	Paediatric age	2/70	2.9	0.015	0.16 (0.04–0.69)
	Diabetes	6/19	31.6	0.002	5.08 (1.21–21.34)
	Carbapenem use	2/51	3.9	0.014	0.18 (0.03–0.95)

* *n*/Total is the number of that particular species found in the total number of patients with the risk factor.

Table 3

Invasive *Candida* species from Pakistan (188 isolates): frequencies and antifungal susceptibility profiles Caspofungin represents echinocandin susceptibilities (caspofungin, anidulafungin, micafungin). The uncommon *Candida* species included *C. pelliculosa*, *C. viswanathii*, *C. utilis*, *C. kefyr*, *C. rugosa*, *C. fabianii* and two novel *Candida species*.

Species	*n* (%)	Antifungal	MIC range (μg ml^{-1})	MIC_{50} (μg ml^{-1})	MIC_{90} (μg ml^{-1})	Resistant/non wild-type (%)
C. tropicalis	61 (32.5)	Caspofungin	0.015–0.12	0.03	0.06	0.0
		Fluconazole	0.25–1.0	0.5	0.5	0.0
		Voriconazole	0.015–0.12	0.015	0.06	0.0
		Itraconazole	0.03–1.0	0.5	1.0	19.7
		Amphotericin B	0.047–0.75	0.25	0.38	0.0
C. albicans	38 (20.2)	Caspofungin	0.15–0.06	0.03	0.06	0.0
		Fluconazole	0.12–2.0	0.25	1.0	0.0
		Voriconazole	0.008–0.12	0.015	0.06	0.0
		Itraconazole	0.06–0.5	0.12	0.25	23.7
		Amphotericin B	0.012–0.125	0.064	0.094	0.0
C. parapsilosis	28 (15.0)	Caspofungin	0.06–1.0	0.25	0.5	0.0
		Fluconazole	0.25–4.0	0.5	1.0	0.0
		Voriconazole	0.008–0.12	0.015	0.06	0.0
		Itraconazole	0.06–0.5	0.12	0.5	0.0
		Amphotericin B	0.016–0.125	0.06	0.125	0.0
C. metapsilosis	4 (2.1)	Caspofungin	0.12–0.25	–	–	–
		Fluconazole	1.0–2.0	–	–	–
		Voriconazole	0.015	–	–	–
		Itraconazole	0.12–0.5	–	–	–
		Amphotericin B	0.032–0.38	–	–	–
C. orthopsilosis	3 (1.6)	Caspofungin	0.12–0.25	–	–	0.0
		Fluconazole	0.5	–	–	0.0
		Voriconazole	0.015–0.03	–	–	0.0
		Itraconazole	0.12–0.25	–	–	–
		Amphotericin B	0.012–0.047	–	–	–
C. glabrata	20 (10.6)	Caspofungin	0.03–0.5	0.03	0.06	5.0

Species	*n* (%)	Antifungal	MIC range (μg ml^{-1})	MIC$_{50}$ (μg ml^{-1})	MIC$_{90}$ (μg ml^{-1})	Resistant/non wild-type (%)
		Fluconazole	1.0–256.0	8.0	64.0	15.0
		Voriconazole	0.06–4.0	0.25	1.00	5.0
		Itraconazole	0.25–>16.0	0.5	1.3	10.0
		Amphotericin B	0.06–0.5	0.25	0.5	0.0
C. guilliermondii	13 (6.9)	Caspofungin	0.03–0.5	0.5	0.5	0.0
		Fluconazole	2.0–4.0	2.0	4.0	0.0
		Voriconazole	0.03–0.12	0.06	0.125	0.0
		Itraconazole	0.25–1.0	0.5	0.5	0.0
		Amphotericin B	0.016–0.064	0.03	0.06	0.0
C. krusei	4 (2.1)	Caspofungin	0.12–0.25	–	–	0.0
		Fluconazole	4.0–32.0	–	–	100.0
		Voriconazole	0.12–0.25	–	–	0.0
		Itraconazole	0.25–1.0	–	–	0.0
		Amphotericin B	0.38–0.75	–	–	0.0
C. lusitaniae	4 (2.1)	Caspofungin	0.25–0.5	–	–	0.0
		Fluconazole	0.25–0.5	–	–	0.0
		Voriconazole	<0.008–0.008	–	–	0.0
		Itraconazole	0.25–0.5	–	–	0.0
		Amphotericin B	0.016–0.064	–	–	0.0
Uncommon *Candida* species	13 (6.9)	Caspofungin	0.015–1.0	0.06	0.5	–
		Fluconazole	0.25–8.0	1.0	4.0	_*
		Voriconazole	<0.008–0.25	0.03	0.25	–
		Itraconazole	0.03–0.5	0.5	0.5	–
		Amphotericin B	0.016–0.5	0.094	0.5	–

*One *C. pelliculosa* isolate was categorized as non-wild-type in respect to fluconazole, as the MIC of fluconazole fell beyond the wild-type range.

Table 4

Susceptibilities and clinical details of rare *Candida* isolates Caspofungin represents echinocandin susceptibilities (caspofungin, anidulafungin, micafungin). The breakpoints for echinocandins and/or fluconazole were not established. CVC, Central venous catheter; HCC, hepatocellular carcinoma; PICC, peripherally inserted central catheter; TACE, transcatheter chemo-embolization; TPN, total parenteral nutrition.

Species	Frequency (*n*)	Source	Patient characteristics	MIC range (μg ml^{-1})				
				Caspofungin	Fluconazole	Voriconazole	Itraconazole	Amphotericin
C. viswanathii	3	Blood culture	28-Year-old male with renal transplant	0.03–0.12	0.5–4.0	0.015–0.25	0.03–0.5	0.032–0.094
			70-Year-old diabetic female					
C. pelliculosa	3	Two blood cultures, one CVC tip	28-Day-old male (history not available)	0.015–0.03	0.25–8.0	<0.008–0.25	0.06–0.5	0.016–0.5
			13-Year-old female with malignancy and CLABSI					
			43-Year-old male with CLABSI and complicated abdominal infection					
C. utilis	2	Blood cultures	Two neonates with late-onset neonatal sepsis	0.015–0.5	1.0	0.03–0.06	0.25	0.032–0.047
Novel *Candida* species	2	Blood cultures	18-Month-old female with burn wound infection on TPN; 28-year-old male on chemotherapy; both received multiple antibiotics	0.06–0.25	0.5–4.0	0.015	0.5	0.047–0.38
C. fabianii	1	Blood culture	Neonate; clinical information not available	0.03	0.5	0.03	0.5	0.094
C. kefyr	1	Blood culture	65-Year-old male; HCC, post-TACE with abdominal abscess and PICC line for chemotherapy	0.12	0.5	0.03	0.5	0.38
C. rugosa	1	Blood culture	70-Year-old female with knee replacement, bed sores and Alzheimer's disease	1.0	1.0	0.008	0.5	0.75