EPIDEMIOLOGY AND RISK FACTORS FOR CANDIDAEMIA AT CHRIS HANI BARAGWANATH HOSPITAL (2009 – 2010)

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DECLARATION

I, Sharona Seetharam, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Microbiology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

_____

___30th___ day of __May___ 2017 in ___Boskruin____

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ABSTRACT

Background

Invasive *Candida* infections (ICI) have emerged as an important cause of increased morbidity and mortality in specific patient populations in recent years. Multiple risk factors coupled with changes in epidemiology have made clinical management of these patients challenging. A laboratory-based surveillance project, Tracking Resistance to Antifungal drugs for *Candida* species in South Africa (TRAC-SA) was conducted at Chris Hani Baragwanath Hospital (CHBH) from 2009 to 2010 and allowed for collection of laboratory information related to episodes of candidaemia, delineation of the situation at the hospital and distribution of information to relevant stakeholders to help make informed clinical decisions.

Objective

Determine the clinical epidemiology and risk factors for bloodstream *Candida* infection at CHBH over an 18-month period

Methods

A retrospective, cross-sectional analysis was carried out on cases of blood culture-confirmed candidaemia from inpatients from 1 February 2009 until 31 August 2010. These cases were identified from the TRAC-SA database, inpatient files were traced and clinical data recorded on a standard case report form. Additional laboratory data of selected tests done within 72 hours of the initial blood culture were obtained from the National Laboratory Health Service Corporate Data Warehouse (CDW).

Results

A total of 167 episodes of candidaemia were identified during the study period with an incidence of 2.09 per 1000 admissions. The distribution of episodes occurred among 55 children (33%), 41 adults (25%) and 71 neonates (43%). The overall species distribution was *Candida* species other than *C. albicans* (98/167, 58.7%) and *C. albicans* (69/167, 41.3%). *Candida* species other than *C. albicans* comprised mainly of *C. parapsilosis* (73/167, 43.7%), *C. glabrata* (10.2 %, 17/167) and other species combined including *C. tropicalis* and *C. krusei* (8/167, 4.7%). Factors associated with *C. albicans* (versus *Candida* species other than *C. albicans)* infection included older age, use of 2 or more antibiotics, use of broad spectrum antibiotics specifically meropenem, aminoglycosides, vancomycin, co-trimoxazole and mechanical ventilation ($p < 0.001$). The overall case-fatality was 59/163 (35.3%). The highest case fatality was noted among adults with *C. albicans* infection, i.e. 15/22 (68.18%).

Significant risk factors associated with in-hospital mortality were use of central lines, urinary catheters, total parenteral nutrition, 2 or more antibiotics, beta lactam - beta lactamase inhibitors, proton pump inhibitors, aminoglycoside and abdominal surgery ($p < 0.01$). Of the *C. parapsilosis* isolates tested, 40 (57. 9%) tested non-susceptible to fluconazole. Risk factors associated with fluconazole resistance included neonatal age, involvement of the respiratory system, mechanical ventilation, chemotherapy, use of a prior antifungal agent and use of 2 or broader spectrum antibiotics ($p<0.01$). Of 71 neonates, 16 (22.5%) received empiric antifungals, in comparison to children (5/55, 9.0%) and adults (4/41, 9.7%) ($p = 0.272$).

Conclusion

CHBH had a high incidence of candidaemia with a predominance of *Candida* species other than *C. albicans* especially in the neonate age group. Risk stratification of in-patients is of paramount importance in choice of empiric antifungal drug due to the differing azole resistance patterns observed.

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1 Chapter 1

1.1 Introduction

1.1.1 General

Candida species are components of the commensal mucosal flora and skin among healthy human beings. They have the potential to cause infection of varying severity, ranging from localised to invasive *Candida* infections (ICI) with either secondary or multi-organ involvement. With advances in medical therapy, such as organ transplantation, life prolongation measures as well as an increase in immunocompromising diseases, the at-risk population and frequency of fungaemia has increased over the past decade (Shorr et al., 2009). In addition, individual risk factors such as extremes of age, low birth weight, use of invasive devices, broad spectrum antibiotics (Cotten et al., 2006), multiple site colonisation (Manzoni et al., 2006) and associated prolonged hospital stays (Blyth et al., 2009; Menzin et al., 2009; Zaoutis et al., 2005) have contributed to this increase.

1.1.2 Morbidity and mortality due to candidaemia

The mortality and morbidity associated with ICI is high. Crude and attributable mortality rates range from 11.9% (Blyth et al., 2009; Zaoutis et al., 2010; Gokcebay et al. 2016)) to 32.1% respectively among paediatric patients to 39%-71% and 49%-81% respectively among adults (Colombo et al., 2006; Eggimann et al., 2003; Gudlaugsson et al., 2003; Horn et al., 2009; Morgan et al., 2005; Pfaller and Diekema, 2007; Wey et al., 1988; Zaoutis et al., 2005). Attributable mortality in these studies was associated with nosocomial candidaemia, and subsequent increased length of stay, while higher mortality was noted in patients who had invasive devices like central catheters. Other contributory risk factors for both higher crude and attributable mortality rates in ICI include the species of *Candida* isolated (Hawkshead et al., 2016), fluconazole resistance and virulence, (Abbas et al., 2000; Bliss et al., 2012; Viscoli et al., 1999)**,** the underlying disease or intervention (Garnacho-Montero et al., 2010; Gokcebay et al. 2016) and the timing as well as choice of antifungal therapy (Savage et al, 2015). In contrast, fluconazole prophylaxis among high-risk infants has been shown to improve mortality rates and reduce ICI (Kaufman, 2003; Leibovitz, 2012; Manzoni et al., 2007). Morbidity is also considerable among infants, especially those with low birth weights and meningitis, with rates of up to 72% of neurodevelopmental disability post-ICI (Benjamin et al., 2010; Brian Smith et al., 2005, Barton et al.,2017). Associated hospital costs and

resource utilisation also escalates in relation to treatment of ICI. (Arendrup, 2010; Craver et al., 2010; Hassan et al., 2009; Morgan et al., 2005; Smith et al., 2007; Zaoutis et al., 2010). Multiple risk factors are shown to be associated with significant cost increases. These include initial treatment failure, length of stay prior to starting antifungals, fever and proven candidaemia (Armanganidis et al., 2017). Risk stratification of patients and early initiation of appropriate antifungal management has been shown to have a significant mortality benefit and cost reduction. (Eggimann and Ostrosky-Zeichner, 2010; Garey et al., 2006; Garnacho-Montero et al., 2010; Morrell et al., 2005; Playford et al., 2010). An analysis of the local epidemiology including resistance rates, antifungal use patterns and associated cost would allow for an appropriate containment strategy to be implemented (Aysegul et al. 2016).

1.1.3 Epidemiological shift in species distribution

In the past decade, epidemiological shifts in species distribution have been noted in different parts of the world, with increasing proportions of *Candida* species other than *C. albicans* isolated from blood cultures (Falagas et al., 2010; Guinea et al., 2014; Goncalves et al., 2016). Multiple surveillance studies carried out in different parts of the world, showed temporal differences in species distribution and susceptibility depending on geographical area, hospital units and between adult, paediatric and neonatal patient populations.

The Artemis DISK Global Antifungal Surveillance Study (1997 – 2003) was one of the biggest such programmes made up of 127 sites in 39 countries and included more than 140000 isolates (Pfaller et al 2005, Guinea 2014). Overall the frequency of *C. albicans* was shown to be decreasing in spite of it being the most common species isolated worldwide. *Candida* species other than *C. albicans* such as *C. glabrata* were most often seen in the USA and Northern Europe rather than *C. parapsilosis*. In contrast, the converse was more common in Brazil (Guinea 2014). This increase in *C. glabrata* has been observed in patients of increasing age prompting questions regarding mucosal immunity and colonisation in the aging population (Pfaller et al. 2002). Local hospital factors e.g. azole use and infection control protocols were postulated as significant variables which could impact on species distribution (Guinea et al., 2014; Goncalves et al., 2016). Nosocomial spread of infection could also account for the larger proportions of *C. albicans* and *C. parapsilosis* isolated in certain populations like neonates or patients in oncology. (Escribano et al. 2013; Asmundsdottir et al. 2008). Possible reasons for this include the increased use of interventions

like central venous catheters and long term indwelling ports in these patients and poor infection control.

The Artemis and Sentry Antimicrobial Surveillance programme (1997- 2002) also examined susceptibility patterns amongst isolates (Pfaller et al 2002). With changes in species distribution, azole non-susceptibility associated with species such as *C. parapsilosis* and *C. kefyr* was shown to be increasing. *C. albicans* demonstrated no consistent changes to fluconazole with overall *C. glabrata* demonstrated variable susceptibility patterns based on geographic region with rates of 10.6% in Asia-Pacific, 13.0% in Latin America and 16.5% in Europe. Differences between sources of ICI, as well as the emergence of less common species like *C. krusei* which exhibit fluconazole resistance, have had implications for prophylaxis and empiric therapy (Pfaller et al., 2011b) especially in high-risk groups (Pfaller et al., 2010). Increased azole use has been implicated in changes in species distribution. Variable fluconazole susceptibility in species like *C. parapsilosis* have been noted in countries like Brazil and South Africa where more resistant organisms have been associated with outbreaks (Goncalves et al. 2016). Resistant organisms also place limitations in the usage of available antifungals (Govender et al., 2016). With increasing use of echinocandins, baseline susceptibility profiles are important in determining trends in resistance. Low levels of resistance to all available echinocandins was noted except for *C. glabrata* isolates from North American isolates (Pfaller et al. 2010).

Emergence of new resistance mechanisms and multidrug resistant fungi leading to breakthrough infections highlighted the need for long term monitoring of susceptibility profiles as well as emphasising the need for newer, molecular methods to determine mechanisms of resistance. An example of one such fungal pathogen is *C. auris*, first described in Japan in 2009. It has now emerged globally as a healthcare-associated pathogen which has demonstrated resistance to multiple antifungal drug classes, difficult to identify in the laboratory and has the potential for nosocomial transmission. Patient populations involved are not limited to critically ill , but also those with haematological conditions (Vallabhaneni et al 2016) In light of this, identification of variables potentially associated with fluconazole non susceptibility (Garnacho-Montero et al., 2010) or multi antifungal drug resistance are of paramount importance for initiating appropriate timeous management of patients.

Different therapeutic strategies utilised for ICI has also had variable impact on species distribution and resistance patterns especially in ICUs. As *Candida* colonisation and ICI occurs along a continuum, choice of initial antifungal has been based on factors like number

of body sites colonised, co morbid conditions, biomarkers and previous drug exposure. The China -SCAN (China Survey of candidiasis in the ICU) and Amarcand2 study demonstrated a decrease in early hospital mortality in patients empirically commenced on an antifungal (Na Cui et al. 2017, Montravers et al. 2017). The majority of these participants were critically ill patients who cultured azole susceptible *Candida* isolates from sterile cultures. In contrast, the EMPIRICUS (Empirical Antifungal Treatment in ICUs) (Timsit et al., 2017) and MSG-01 trial (Ostrosky-Zeichner et al. 2017), both used echinocandins empirically in critically ill patients with no significant change in mortality at 28 days. *Candida* colonisation indices and 1, 3 beta-D (B-D) glucan assay, among other criteria, were used to try and risk stratify patients, in EMPIRICUS. Although these have been have been previously shown to be useful in initiating therapy as well as lowering rates of ICI without affecting species distribution, the lack of impact on mortality implies that the either the clinical risk factors play a more significant part in choice of empiric antifungal or that the biomarkers used were insufficient.

Pre-exposure to azoles and echinocandins has also been shown to decrease the prevalence of *C. albicans* in favour of less antifungal susceptible species like *C. glabrata* (Lotholary et al. 2011). Consistent use of blind empiric therapy without documented cultures, would have not just selective pressure but also cost implications and affect species distribution.

1.1.4 Laboratory-based diagnosis of candidaemia

Timeous diagnosis of candidaemia is critical in optimising therapy and improving the outcome. Additionally, isolation of yeasts from sterile specimens such as tissue and blood cultures has been relied upon for the diagnosis of fungaemia. However, numerous problems associated with blood culture-taking practices contribute to yields as low as 6% (Kosmin and Fekete, 2008), as well as the possibility of negative yields even in the presence of disseminated fungaemia of up to 50% (Berenguer et al., 1993). Histological demonstration of tissue invasion can provide a diagnosis. However, the risk associated with obtaining a representative specimen must be taken into account, especially in paediatrics.

Current microbiology-based diagnostics still consist of conventional microscopy and culture-based methods. Blood cultures taken for invasive candidaemia are limited by the delay due to incubation and slow turnaround time. When positive, microscopy usually consisting of a Gram stain of a yeast, can provide a rapid diagnosis identification and aid in initiation of empiric therapy and search for complicated infection. Histological or microscopy techniques can be used to rapidly identify fungal elements for a provisional diagnosis. However,

definitive diagnosis relies on identification on either phenotypic or biochemical characteristics or both. Since this can take from 24 to 72 hours depending on the method (manual or automated) used, it leads to delays in commencing antifungal therapy. Development of chromogenic culture media and newer technology like the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) have enabled faster identification of organisms. The MALDI-TOF is based on analysis of protein patterns that are compared to a database for species level identification. It has been used to rapidly identify isolates both from culture as well as directly from blood cultures (Pulcrano et al. 2013; Rizzato et al. 2015). However, cost of the equipment at the outset is currently prohibitive for the public sector in South Africa.

Non-culture based methods utilising cell wall components, fungal DNA, fungal protein or surrogate markers of infection, are also applicable in patient management to provide prognostic information and/or permit therapeutic monitoring. Of these technologies, the 1, 3 beta-D (B-D) glucan assay is available for routine use currently in South Africa in the public and private sector. Application of the 1, 3 beta-D (B-D) glucan assay has been mostly in certain at-risk groups such as haematology or oncology patients (Odabasi et al., 2004). However, there are still uncertainties with regards to use in paediatrics, as well as variability in performance depending on technical and host factors (Budhavari, 2009; Alexander and Pfaller, 2006). Recent studies have looked at the sensitivity of the assay in relation to the species implicated. This was found to be most sensitive with *C. albicans* and the lowest levels are seen in *C. parapsilosis* (Mikulska et al. 2016). Ideally, the 1, 3 beta-D (B-D) glucan assay can be used in patients at risk for candidaemia with follow up culture or non-culture based methods used for definitive identification of isolates for those with positive tests.

Other biomarkers like procalcitonin (PCT), which is produced in response to infection, has been evaluated for the diagnosis of ICI. A large case control study by Peiralli et al (2017) examined cases of ICI in critically ill patients admitted to internal medical units and the associated PCT values. The conclusion was that PCT values greater than 2.5ng/ml had high negative predictive values for ICI when compared to bacterial infections.

Various molecular techniques are available, mostly in the research or reference laboratory setting. The use of DNA sequencing has helped not only in species identification but also in detection of resistance mechanisms (Goncalves et al. 2016; Sidiq et al. 2016).

1.1.5 Role of surveillance programmes

Ideally surveillance programmes should be able to not only provide information regarding temporal and geographic trends in species distribution, but also demonstrate and help predict

trends in antifungal susceptibility and identify risk factors for invasive candidaemia. Design of the ideal surveillance programme should incorporate the following characteristics: long term study design on a global or national scale; standardised methodology for participating laboratories; provide confirmatory reference testing at central laboratories; use a central database; and have procedures in place to validate the data sent in by participating site.

Programmes, such as the international Sentry Antimicrobial Program, have played a vital role in defining epidemiology, species distribution and changing trends in antifungal susceptibility patterns (Arendrup et al., 2011; Colombo et al., 2006; Pfaller and Diekema, 2002). Previously undefined risk factors have emerged as important entities. These include associations between the species isolated, and community-onset candidaemia (Pfaller et al., 2011c), age-related species distribution (Pfaller et al., 2010; Wisplinghoff et al. 2013; Hawkshead III et al., 2016), fluconazole resistance (Pfaller et al., 2011a) and geographic variation (Pfaller et al., 2011b).

Studies encompassing the above traits are necessary to establish prevalence of both common and rare species and resistance phenotypes. These could then be tracked to determine trends, pre-empt and guide selection of antifungals. However, most of these studies are laboratory-based with limited clinical data which would help in defining management of ICI in the local setting.

1.1.6 The South African perspective

Considering the globally changing geographic trends in species distribution and antifungal resistance, local national surveillance studies are needed to delineate and compare trends between provinces and the different health provision sectors i.e. private and public. These would also provide information regarding antifungal susceptibility and thus contribute to establishing specific treatment and preventative strategies including infection control.

Local literature to date looks at both single and multi- centre studies detailing *Candida* epidemiology (Arendse and Orth, 2008; Badenhorst et al., 1991). Although the Kreusch study was based at CHBH over a 13 year period and was the first to delineate *Candida* epidemiology in the hospital, it was limited by the inclusion of only adult patients (Kreusch et al, 2013). The distribution of isolates was different between the above-mentioned studies: *Candida* species other than *C. albicans* dominated in paediatrics while *C. albicans* was prominent in adults. The surgical ICU and the general wards were the commonest settings for infections. Risk factors for candidaemia included abdominal surgery, HIV infection, diabetes mellitus, use of foreign devices and exposure to broad spectrum antibiotics (Kreusch et al,

2013). The only study describing resistance patterns was by Govender et al where a worrying trend of azole non-susceptible *C. parapsilosis* isolates from both private and public sector hospitals limited choice of antifungal therapy in this resource limited setting (Govender et al, 2016).

From 1 February 2009 until 31 August 2010, Phase 1 of a laboratory-based sentinel surveillance project, TRAC, (Tracking Resistance to Antifungal drugs for *Candida* species in South Africa) was carried out in collaboration with 11 university affiliated or regional public-sector hospitals and ≥85 private sector hospitals served by the 5 largest amalgamated private pathology practices across South Africa. The major objectives of this project were to: describe the species distribution of *Candida* spp. causing bloodstream infection at sentinel sites in South Africa, compare the species distribution and describe the prevalence of resistance to nine antifungal drugs (including fluconazole, voriconazole, amphotericin B, caspofungin). During that period, 407 cases of ICI were recorded from CHBH, the largest number of cases from any participating public-sector hospital in South Africa.

This is the first study, nested within TRAC-SA surveillance, to characterise the clinical epidemiology of invasive candidaemia at CHBH in both adult and paediatric patients and to evaluate the risk factors and features associated with azole resistance.

1.2 Specific aim

To describe the clinical epidemiology and risk factors for candidaemia at CHBH from February 2009 through August 2010

1.3 Objectives

1.3.1 Primary objective

To describe the epidemiology of laboratory-confirmed invasive candidaemia at CHBH from 1 February 2009 until 31 August 2010.

1. To describe the demographics of hospitalised patients with laboratory-confirmed invasive candidaemia
2. To describe the clinical management of patients with laboratory-confirmed invasive candidaemia with regard to antifungal therapy

3. To describe the species distribution and antifungal drug resistance

1.3.2 Secondary objectives

1. To describe clinical risk factors for patients with lab-confirmed invasive candidaemia
2. To describe the clinical risk factors for fluconazole-resistant *Candida* infection
3. To compare the epidemiology of patients with fluconazole-susceptible and fluconazole-resistant invasive *Candida* infection

1.4 Methods

This cross sectional retrospective study aimed to describe the clinical epidemiology and risk factors for candidaemia from inpatients between 1 February 2009 until 31 August 2010 admitted to Chris Hani Baragwanath Hospital (CHBH) in Soweto, Gauteng, South Africa. This is an academic hospital with a bed capacity of approximately 3200. It offers a number of specialist services including oncology, infectious diseases, neurosurgery, intensive care (neonatal, paediatric, surgical), trauma and general medicine facilities.

The hospital was a participant of the Tracking Resistance to Antifungal drugs for *Candida* species in South Africa (TRAC – SA) surveillance system. This was national laboratory based surveillance intended to describe characteristics of episodes of candidaemia amongst patients at sentinel sites in the private and public health sectors in South Africa from 1 February 2009 to 31 August 2010. It included all incident episodes of blood culture confirmed candidaemia from any patient during the specified time period.

Basic laboratory information regarding species identification and limited antifungal susceptibility on isolates obtained at the CHBH laboratory was submitted with the isolate to the Centre for Opportunistic, Tropical and Hospital Infections (COTHI) at the National Institute for Communicable Diseases (NICD) in South Africa for microbiological confirmation, antifungal susceptibility testing and further characterisation if necessary. The episode was logged on the TRAC SA database as an episode. Of the episodes with mixed cultures i.e. more than 1 species isolated, the most resistant isolate was included in the analysis. The mixed cultures identified from the blood culture bottle at the reference laboratory were numbered e.g. 123A, 123B and matched a single CHBH laboratory number.

1.4.1 Definitions

An incident episode was defined as the first isolation of a *Candida* species from a patient's blood culture. Any episode occurring more than 30 days (definition based on previously published studies) thereafter was classified as a new episode. If subsequent positive blood cultures for *Candida* species were drawn from the same person >30 days after the first isolation, the first of these cultures defined a recurrent episode of candidaemia for that individual. For this study, patients were classified according to their admitting sections i.e. neonates either born at CHBH or transferred from other hospitals are admitted to the Neonatal unit, paediatrics (<18 years) and adults (≥ 18 years). The neonatal unit is comprised of 3 units – Intensive Care Unit which admits patients requiring ventilation, high care or transitional care unit (babies not requiring ventilation but still having central lines, receiving TPN, etc.) and neonatal ward (babies without any central lines admitted for weight gain). These definitions were used as patients were not moved from their admitting sections if they changed age categories during their hospital stay.

For this retrospective file review patients with blood culture-confirmed candidaemia at CHBH were identified from the surveillance database and files were retrieved from medical records. Data was collected on a case report form (Appendix 5.3). This included demographic information, risk factors for candidaemia, concomitant conditions, complications, antifungal therapy, and clinical outcome. Additional laboratory data of selected tests done within 72 hours of the initial blood culture was obtained from the National Laboratory Health Service Corporate Data Warehouse (CDW).

1.4.2 Microbiologic methods

1.4.2.1 CHBH

Standard operating protocols were followed for phenotypic identification and susceptibility testing of *Candida* species at the CHBH laboratory. This consisted of subculture from the blood culture broth onto Sabouraud Dextrose agar (Diagnostic Media Products, National Health Laboratory Service, Johannesburg, South Africa). Species identification was based on germ tube production and the Auxacolor Yeast Identification system (Biorad, France). Antifungal susceptibility testing was done with E tests (bioMerieux, France) for fluconazole and voriconazole on RPMI media (bioMerieux and Diagnostic Media Products). The isolate

was sub cultured onto Dorset transport medium (Diagnostic Media Products) for submission to COTHI.

1.4.2.2 COTHI

Species identification was confirmed using morphologic and biochemical criteria. Isolates were initially inoculated onto chromogenic agar (MAST ID CHROMagar *Candida*, Mast Diagnostic, Amiens, France) to determine whether more than one species was present. Subsequent identification to species level was by one or more of the following biochemical kits or instruments: Vitek 2 YST, API 20C AUX and API ID 32C (bioMerieux, Marcy l'Etoile, France).

Antifungal susceptibility testing for fluconazole, voriconazole, posaconazole, itraconazole, flucytosine, micafungin and anidulafungin was performed using pre-prepared dried microbroth dilution panels containing Alamar blue (Thermo Fisher Scientific, Cleveland, OH, USA). MIC values were determined visually following 24 h of incubation. MICs of amphotericin B were determined by Etest (bioMerieux) on RPMI 1640 plates containing 2% glucose (Diagnostic Media Products). Any isolate with an initial resistant MIC was retested with Etest (bioMerieux).

1.4.3 Statistical analysis

This was performed with STATA version 11(StataCorp LP, USA) and a *p* value of <0.05 was considered significant. Continuous variables were compared with Student's T test and categorical variables compared with Fisher's exact test.

1.4.4 Ethics

Approval for the TRAC-SA surveillance study as well as the retrospective review were obtained from the Human Ethics review committee (Medical), University of the Witwatersrand, Johannesburg (Approval number M120613) and the CHBH hospital management.

2 Chapter 2

2.1 Results

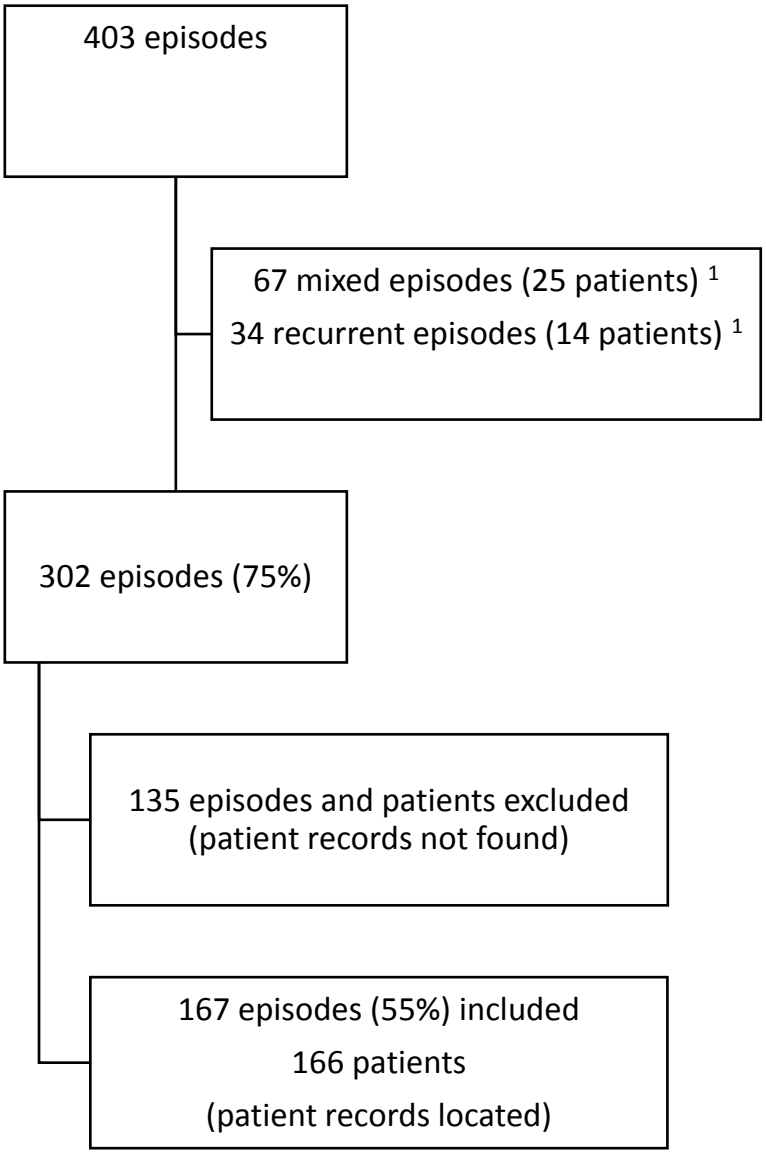

Figure 1. Flowchart of all episodes logged on the database

[1]

Episode – Incident/first culture of *Candida* species from blood culture within a 30 day period.

Mixed – more than 1 species isolated from the same blood culture bottle as per the reference lab

Recurrent – Any episode occurring after the incident culture in a 30 day period

A total of 403 episodes of candidaemia were documented from February 2009 until August 2010. Of these, 166 patients (167 episodes) with completed case report forms were included for analysis (Figure 1). Some blood cultures were obtained at different times, sometimes in multiple sets, and the site was not often stated e.g. peripheral or central line.

Overall, 135 patients were excluded from the study as the records could not be located in the archives and 167 episodes (166 patients) were included. Some patients had both mixed and multiple cultures. Basic demographics obtained from the laboratory of both included and excluded groups are shown below. The age and prior antifungal category showed significant differences between both groups (age, $p < 0.001$; prior antifungal given, $p < 0.001$). For neonates, a significantly higher percentage (>50%) of records could not be located compared to other age groups

Table 1. Demographic characteristics of included (167 files found and included in study) and excluded (135 files not found and excluded from study) patient (302) groups

Characteristic	Totals	Files found n =167 Number (%)	Files not found n=135 Number (%)	p value
Age				**<0.001**
Neonates	168	71 (42.3)	97 (57.7)	
Paediatrics	70	55 (78.6)	15 (21.4)	
Adults	64	41 (64.1)	23 (35.9)	
Gender				0.513
Male	141	75 (53.2)	66 (46.8)	
Female	161	92 (57.1)	69 (42.9)	
Species				0.642
C. albicans	124	69 (55.6)	55 (44.4)	

Characteristic	Totals	Files found n =167 Number (%)	Files not found n=135 Number (%)	p value
Non *C. albicans*	178	98 (55.1)	80 (44.9)	
Fluconazole resistance				
Total	55	41 (74.5)	14 (25.5)	0.853
C. parapsilosis	52	40 (76.9)	12 (23.1)	0.577
Antifungal prior to BC				**<0.001**
Fluconazole	33	27 (81.8)	6 (18.2)	
Amphotericin B	20	15 (75.0)	5 (25.0)	

2.1.1 General patient demographics

Of 167 episodes, 55 occurred in children (33%), 41 in adults (25%) and 71 in neonates (43%). The overall age range for patients was birth to 90 years (median 6 months, interquartile range (IQR) 14.6 days – 12 years). The median age and IQR for each group was: neonates, 15 days (IQR 6-24 days); children, 2 months (IQR 11 months – 10 years) and adults, 44 years (IQR 30-57 years).

Combined, the age group from birth to 4 years of age made up 113 (67.6%) of episodes. Within the neonate group, 62/71 (87.3%) of babies were low birth weight (LBW), i.e. < 2500 g and 68/71 (95.8%) were premature < 37 weeks gestation (median 30 weeks; IQR 27-32 weeks). Of these, 13 (21.1 %) were extremely low birth weight (ELBW) at <1000 g (median 890 g (IQR 785-935g). There was no significant difference in gender distribution of candidaemia (females, 92/167 (55.0%) compared to males, 75/167 (44.9%); p = 0.115)). The proportion of new-born baby boys was 52% (39/75) compared to male children (21/75; 28.0%) and adult males (15/75; 20%) (p=0.051).

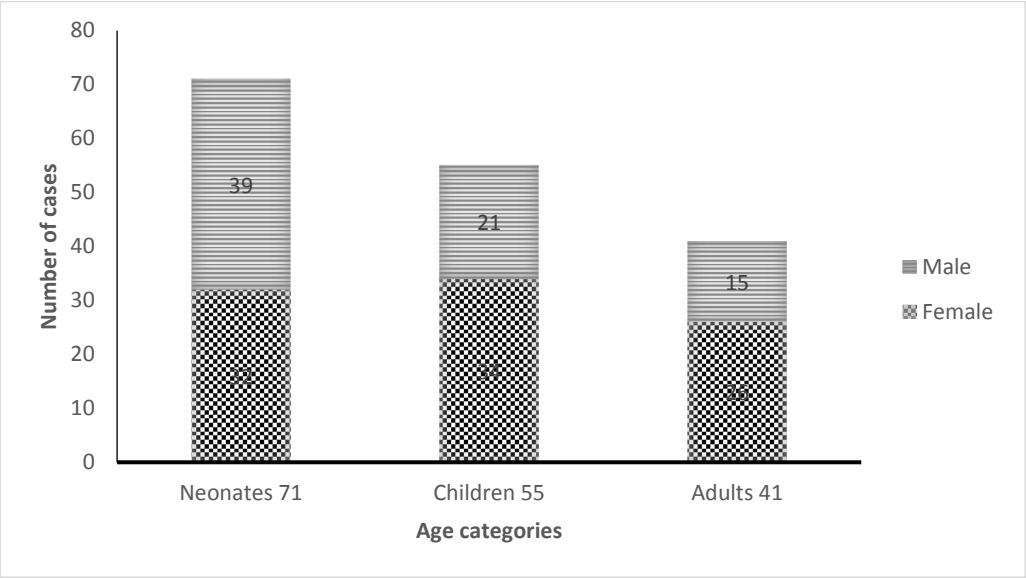

Figure 2. Gender distribution by age category

2.1.2 Distribution of candidaemia within the hospital

The majority of episodes 71/167 (42.5%) occurred in neonates compared to the adult group (41/167; 24.5%) and the paediatric group 55/167(32.9%). A significantly higher number of patients 15/41 (36.5 %) of cases from adult general medicine (Figures 2 and 3) compared to adult ICU (10/41; 24.4%) (p= 0.031). There were significant differences in species distribution within the age groups. Within the adult group, species distribution varied with *C. albicans* predominant *(*11/15; 73.3%) in general medicine, *C. parapsilosis* (5/9; 55.6%) in general surgery and *C. glabrata* (5/10; 50.0%) in ICU (p= 0.002). The neonatal unit had a different distribution with *C. parapsilosis* being the commonest in the unit (42/71, 59.1%), while *C. albicans* was predominant in the paediatric general medical units (13/29; 44.8%) (p= 0.002).

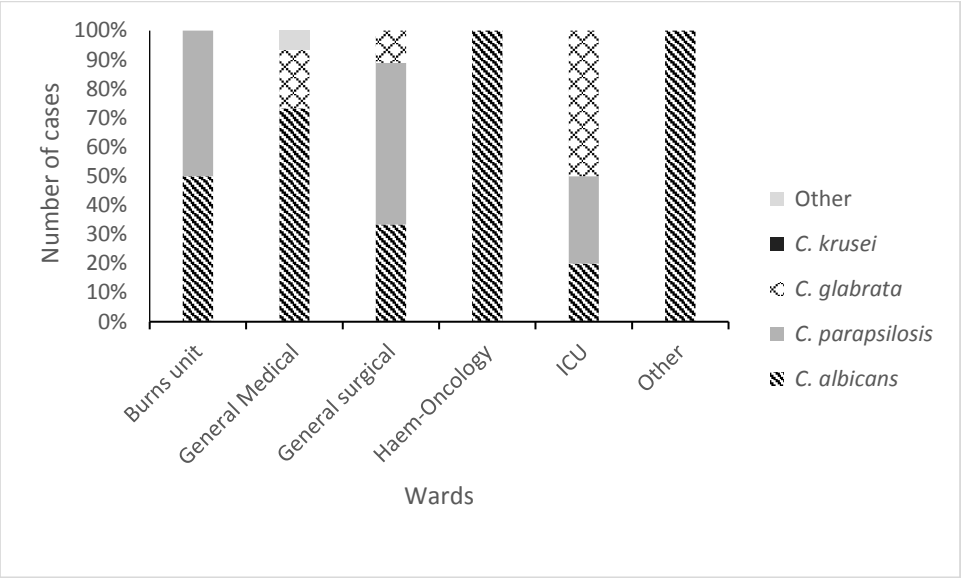

Figure 3. Species distribution in adult wards

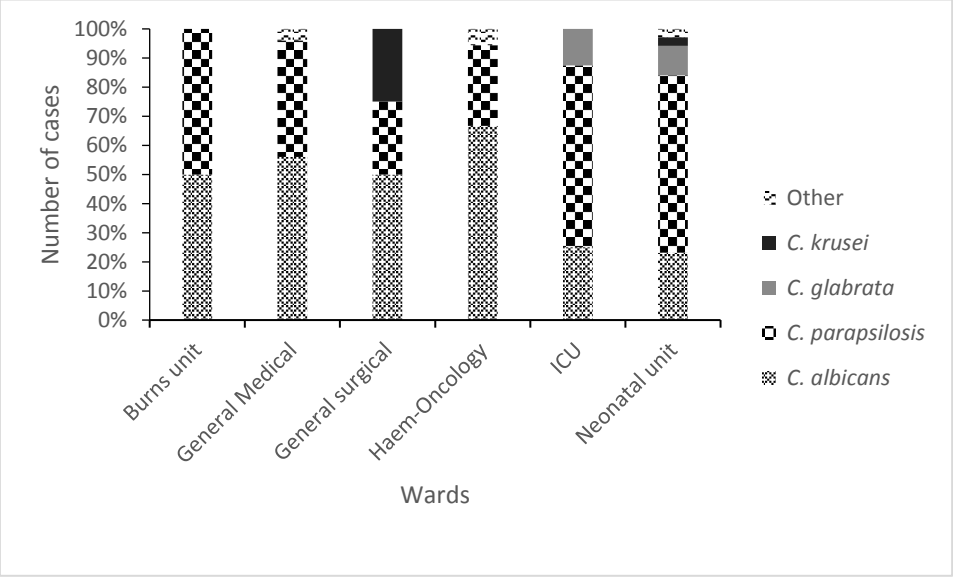

Figure 4. Species distribution in paediatric and neonatal wards

2.1.3 Incidence and in-hospital parameters

Incidence of candidaemia was highest in the paediatric ICU (3.13/100) admissions followed by neonates (2.90/100 admissions). The median length of stay of any inpatient was 31 days (IQR 12-48 days) while the median time spent in hospital prior to the first positive blood

culture was 13 days (IQR 4-22 days). The median length of stay for patients with non *C. albicans* infections was 44 days compared to 34.4 days for *C. albicans* infections (p=0.318). Comparisons between neonates and paediatrics, and neonates and adults of overall length of stay and length of stay prior to the first positive blood culture are shown in Table 2.

Table 2. Total length of stay in hospital and length of stay prior to positive blood culture per age category

	Neonates Total 71	Paediatrics Total 55	Adults Total 41	p value
Overall length of stay (Median, IQR)	37 days, 20-62 days	26 days, 10-43 days	19 days, 10-36 days	*N vs. P p= 0.009 P vs. A p= 0.375 N vs. A p= 0.002
Length of stay prior to positive blood culture (Median, IQR)	15 days, 7-23 days	9 days, 1-16 days	13 days, 1-22 days	N vs. P p= 0.02 P vs. A p= 0.573 N vs. A p= 0.211

*N – neonates; P- paediatrics; A-adults

2.1.4 Mortality

Outcome was known for 163 patients. Of these, 59 (35.3%) patients died with 28/59 (47.4%) being adults. The species isolated in this age group was significant: *C. albicans* was cultured from 15/28 (53.57%) adults and 7/13 (53.84%) paediatric patients who died while in neonates 11/18 (61.1%) *C. parapsilosis* was isolated (P = 0.006). Case fatality ratios were not significantly different in adults admitted to the general medical wards [(12/28, 42.8 %) compared to those admitted in ICU (10/28; 35.7%) (P = 0.123). Of 59 patients who died, 35 (59.3%) died within 7 days of admission. Of 37/166 patients with either confirmed HIV or HIV exposure, 13/37 (35.1%) died compared to 129/166 (77.7%) of HIV negative patients. (p=0.532) The CD4 count was available for 14 HIV-seropositive patients, 7 of whom had advanced immunosuppression with CD4 < 200 (median CD4 count 198; IQR 33-975).

Table 3. Case fatality ratios based on species and age category

Species	Neonates	Paediatrics	Adults
	Number demised/total isolates (%)		
C. albicans	6/18 (33.33)	7/29 (24.14)	15/22 (68.18)
C. parapsilosis	11/42 (26.19)	4/22 (18.18)	3/9 (33.33)
C. glabrata	1/7 (14.29)	1/1 (100.0)	9/9 (100.0)
C. krusei	0/2 (0)	0/1 (0)	0/0 (0)
Other*	0/2 (0)	1/2 (50.0)	1/1 (100.0)

*Includes *C. krusei, C. tropicalis, C. lusitaniae*

Table 4. Univariate analysis of risk factors for mortality

Risk factors	Total with intervention	Number demised	Number survived	p value	95% CI	OR
Mechanical ventilation	80	34	46	0.101	0.93 - 1.00	0.97
Central lines	119	53	66	**0.001**	0.85 - 0 .95	0.90
Urinary catheter	40	24	16	<**0.001**	0.88 - 0.96	0.92
Total parenteral nutrition	9	7	2	**0.019**	0.83 - 0.98	0.90
Abdominal surgery	34	18	16	**0.025**	0.92 - 0.99	0.95

Risk factors	Total with intervention	Number demised	Number survived	p value	95% CI	OR
2 or more antibiotics	109	33	76	**0.027**	1.09 - 4.1	2.13
Beta lactam- beta lactamase inhibitor	36	22	14	**0.001**	0.91 - 0.97	0.94
Proton pump inhibitor	7	6	1	**0.025**	0.85 - 0.98	0.91
Aminoglycoside	83	23	60	**0.023**	1.00 - 1.03	1.01
Chemotherapy	15	3	12	0.182	0.98 - 1.06	1.02
Steroids	44	19	25	0.261	0.95 - 1.01	0.98

The significant risk factors on univariate analysis noted for mortality included use of central lines, urinary catheters, total parenteral nutrition, 2 or more antibiotics, beta lactam - beta lactamase inhibitor, proton pump inhibitor, aminoglycoside and abdominal surgery (Figure 3).

2.1.5 Laboratory results

2.1.5.1 Microbiology

2.1.5.1.1 Isolates

Of 167 episodes analysed, *Candida* species other than *C. albicans* (98/167; 58.68%) were more frequently isolated than *C. albicans* (69/167; 41.31%). Within the *Candida* species other than C. *albicans* group. *C. parapsilosis* was the commonest species isolated (73/167; 3.7%) followed by *C. glabrata* at 10.2 % (17/167).

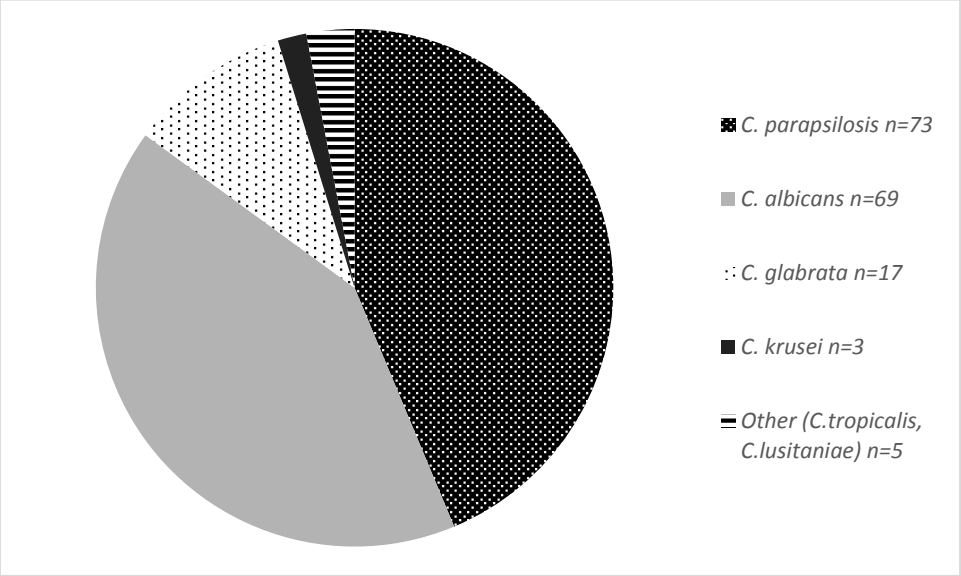

Figure 5. Distribution of species isolated

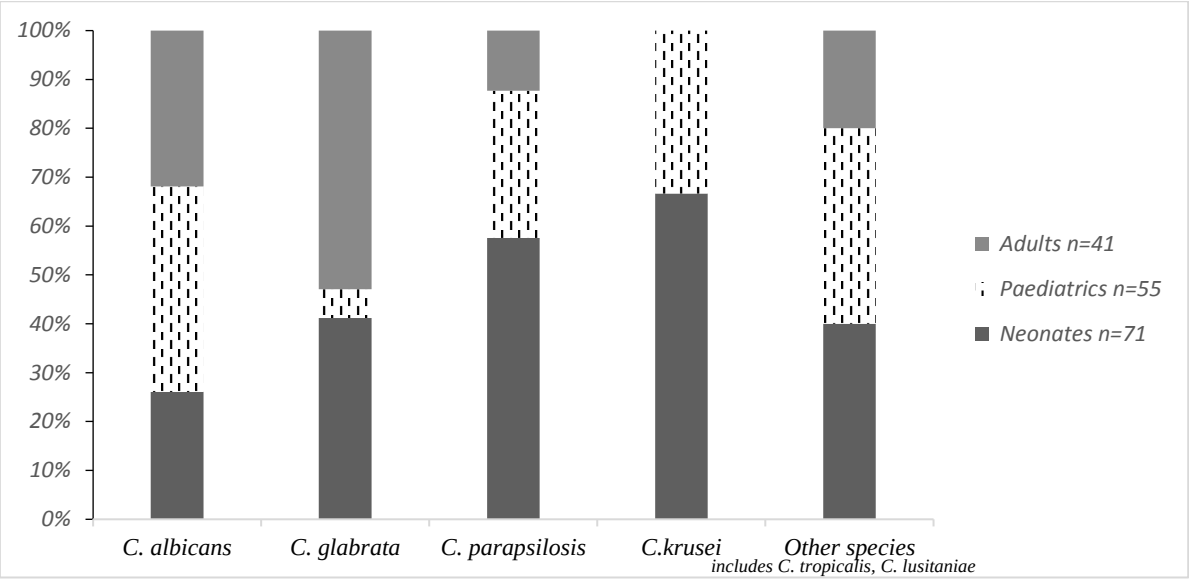

Figure 6. Species distribution per age category

C. parapsilosis isolation was significantly higher in neonates (42/73; 57.5 %) compared to children (22/73; 30.1%) and adults (9/73; 12.3%) (p = 0.002). Adult patients also had the highest number of *C glabrata* at 52.9% (9/17) compared to neonates at 41.1% (7/17) (p=0.003) (Figure 6).

Within the low birthweight groups, 39/62 (48. 39%) of isolates were *Candida* species other than C. *albicans* species specifically *C. parapsilosis*. Of this group, 23/39 (58.9%) were in the VLBW category (< 1500 g). Of the 4 babies who cultured *C. glabrata*, 3 were diagnosed with necrotising enterocolitis during their hospital stay not requiring surgery. Their initial admission was for respiratory distress and all were premature.

At NICD, 13 patients were diagnosed with mixed infections. Of all these episodes, 9 had 3 species identified while the rest had dual species candidaemia. The commonest combinations of organisms were *C. albicans* and *C. parapsilosis*. Of these mixed cultures, the fluconazole MIC reflected that of the most resistant isolate present. This was mainly *C. parapsilosis* with high fluconazole MICs.

2.1.5.1.2 *In vitro* susceptibility

Antifungal susceptibility results for fluconazole and voriconazole were available for 161 and 143 episodes respectively. All *C. albicans* isolates were uniformly susceptible to both drugs. Of the *C. parapsilosis* isolates tested, 40 (57.9%) were non-susceptible to fluconazole with 30 isolates having MICs >256 (high level resistance). Discordant fluconazole susceptibilities

which showed very major errors (category change e.g. susceptible to resistant) was observed for 18 *C. parapsilosis* and 15 *C glabrata* isolates between the reference and originating laboratory. All 40 *C. parapsilosis* isolated tested susceptible to caspofungin, though this is not the recommended agent for in vitro susceptibility testing.

Overall 24/167 (14.3%, p=0.009) of patients received an antifungal either empirically or prophylactically. Neonates 16/71 (22. 5%) received a similar proportion of antifungals in comparison to paediatrics (5/55, 9.0%) and adults (4/41, 9.7%) (p = 0.272). Of the 24 episodes, 21 patients (81. 2%) received fluconazole and 18.7% received amphotericin B. Azole resistant *C. parapsilosis* was isolated in 38 patients. Of these, 10/38 (26.3%) received fluconazole prophylactically/empirically compared to 5/31 (16.1%) fluconazole susceptible *C. parapsilosis* which was not significant (p= 0.06). Overall 141/167 (84.43%) of episodes were treated with an antifungal drug. Amphotericin B was used in 30/44 (68.2%) episodes treated in neonates while fluconazole was used for 32/71 (45.0%), 26/55(47.2%) and 27/41(65.8%) episodes in neonates, children and adults respectively.

Significant risk factors on univariate analysis for fluconazole resistance included falling in the neonate category, involvement of the respiratory system, mechanical ventilation, use of a prior antifungal and use of 2 or broader spectrum antibiotics including beta lactams, cotrimoxazole and aminoglycosides. (Table 5)

Table 5. Risk factors for fluconazole resistance

	Fluconazole						
	Total tested (n)	Susceptible n (%)	Resistant n (%)*	p value		95% CI	OR
Age					**<0.001**	1.65-5.03	2.89
Neonates	69	40 (57,97)	29 (42,03)				
Adults	40	36 (90,00)	4 (10,00)				
Ward					**<0.001**	1.69 5.17	2.96
Neonates	68	39 (57,35)	29(42,65)				
Paediatrics	53	45(84,91)	8(15,09)				
Adults	40	36 (90,00)	4(10,00)				
Systems					0.919	0.92-1.07	0.99
Respiratory	74	48(64,86)	26(35,14)				
Cardiac	5	3(60,00)	2(40,00)				
GIT	31	25(80,65)	6(19,35)				
CNS	8	7(87,50)	1(12,50)				
Skeletal	1	1(100,00)	0(0,00)				
Malignancy Haem	10	10(100,00)	0(0,00)				

Fluconazole						
	Total tested (n)	Susceptible n (%)	Resistant n (%)*	p value	95% CI	OR
Malignancy Solid	13	12(92,31)	1(7,69)			
Burns	4	3(75,00)	1(25,00)			
NEC	5	2(40,00)	3(60,00)			
Renal	1	0(0,00)	1(100,00)			
Metabolic	9	9(100,00)	0(0,00)			
Lines	99	83(83,840	16(16,16)	0.625	0.93-1.03	0.98
Urinary catheter	41	32(78,05)	9(21,95)	0.550	0.96-1.06	1.01
Mechanical ventilation	78	51(65,38)	27(34,62)	**0.011**	0.90-0.98	0.94
Total parenteral nutrition	9	7(77,78)	2(22,220	0.818	0.93-1.09	1.00
Abdominal surgery	35	23(65,710	12(34,29)	0.179	0.93-1.01	0.97
Nosocomial sepsis	32	21(65,630	11(34,38)	0.815	0.97-1.01	0.99
More than 2 antibiotics	107	74(69,160	33(30,840	**0.031**	0.16-0.91	0.38
3rd generation cephalosporin	52	44(84,62)	8(15,380	**0.046**	1.00-1.07	1.03
Beta lactam-Beta Lactamase Inhibitor	37	33(89,190	4(10,810	**0.026**	1.00-1.09	1.05
Fluconazole	25	17(68,000	8(32,00)	0.416	0.95-1.02	0.98
Amphotericin B	14	7(50,000	7(50,00)	**0.035**	0.92-0.99	0.95

	Fluconazole					
	Total tested (n)	Susceptible n (%)	Resistant n (%)*	p value	95% CI	OR
Proton pump inhibitor	7	5(71,430	2(28,57)	0.847	0.93-1.05	0.99
Meropenem	52	33(63,460	19(36,54)	**0.028**	0.94-0.99	0.97
Vancomycin	43	29(67,44)	14(32,56)	0.215	0.96-1.00	0.98
Aminoglycoside	81	54(66,67)	27(33,33)	**0.023**	0.96-0.99	0.98

*Resistant includes susceptible-dose dependent isolates

2.1.5.2 Blood results

Blood tests either done on admission or for a septic workup were C reactive protein (normal value <10 mg/ml), white cell count (WCC) (Range 3.90-12.60 x 10^9/L) and platelet counts (range 186-454 x10^9/L) taken within 72 hours of the incident blood culture (Table 6). The highest CRP and white cell counts were seen in adults (median 166.65, IQR 79.78-213.46 and median 9.75, IQR 7.14-16.95 respectively). The lowest platelet counts were observed in neonates (median 102, IQR 41-249).

1. 3 β- D glucan assays were done for 16 patients within a 72-hour window either prior or subsequent to the blood culture draw. Of these 11/16 (68.7%) were positive. Of the 4/16 (25.0%) were negative and 1/16 (6.2%) was equivocal. Of the positive results, 72.7% (8/11) were neonates who had cultured *C. albicans* and 2/11 (18.18%) cultured *C. parapsilosis*. Negative results (75%, 3/4) occurred in paediatric oncology patients from whom blood had been drawn from the ports and whose blood cultures were positive for *C. albicans*.

Table 6. Laboratory parameters

Laboratory parameters	Total	Neonates N=71	Total	Paediatrics N=55	Total	Adults N = 41	p value
CRP (mg/ml)	55	Median 19.44 (IQR 2.23-16.27)	42	Median 41.28 (IQR 14.74- 79.48)	25	Median 166.65 (IQR 79.78-213.46)	N vs P p=0.006 P vs A p=0.00 N vs A p=0.00
White cell count x (10^9/L)	60	Median 10.61 (IQR 6.34-16.27)	47	Median 6.78 (IQR 1.51-15.43)	34	Median 9.75 (IQR 7.14-16.95)	N vs P p=0.072 P vs A p=0.026 N vs A p=0.884
Platelets (x10^9/L)	60	Median 102 (IQR 41-249)	34	Median 163 (IQR 27-398)	47	Median 186 (IQR 79-417)	N vs P p=0.088 P vs A p=0.348

Laboratory parameters	Total	Neonates N=71	Total	Paediatrics N=55	Total	Adults N = 41	p value
							N vs A p=0.007
1.3 β -D glucan (ug/ml)	5	Median 523 (IQR 500 -523)	7	Median 35.5 (IQR 8-240)	1	Median 523 (IQR 523-523)	N vs P p= 0.008 P vs A p=0.130 N vs A p=0.694

*N – neonates; P- paediatrics; A-adults

2.1.6 Clinical conditions and risk factors

Within neonates and children, the commonest clinical diagnosis or reason for admission involved the respiratory system e.g. pneumonia, with 55/71 (77.4 %) and 17/55 (30.9%) respectively. GIT conditions included trauma and NEC and comprised of 15/41 (36.5%), 9/55 (16.3%) and 12/71 (16. 9%) in the adult, children and neonate groups respectively. There were 24 oncology patients comprising 18/24 (75%) paediatric and 6/24 (25%) adults. This included those with either solid organ involvement or haematological malignancies. Of the paediatric oncology patients, 17/18 (94.4%) were receiving chemotherapy compared to adult oncology patients (2/6; 33.3%) (p <0.001, 95% CI 1.06 - 1.16; OR 1.11)

HIV exposure was noted in 24/71 (33.8%) neonates and diagnosed in 9/55 (16.3%) and 4/41 (9.75%) paediatric and adult patients respectively. The HIV-exposed neonates received antiretroviral drugs as part of the prevention of mother- to-child (PMTCT) HIV transmission programme. Of the 23/24 (95.81%) babies for whom information regarding the mode of delivery was known, there was no significant association between babies delivered by Caesarean section 12/23 (52.1%) and those delivered by normal vaginal delivery 11/23 (47.8%) (p = 0.160). In this HIV exposed group, the species distribution was not significant 14/24 (58.3%) *C. parapsilosis*. 8/24(33.3%), *C. albicans* and 2/24 (8.3%) and *C. glabrata* (p=0.460). At the time of the positive blood culture 29/73 (39.7%) of infections in neonates were classified as nosocomial sepsis i.e. onset of sepsis more than 72 hours after admission.

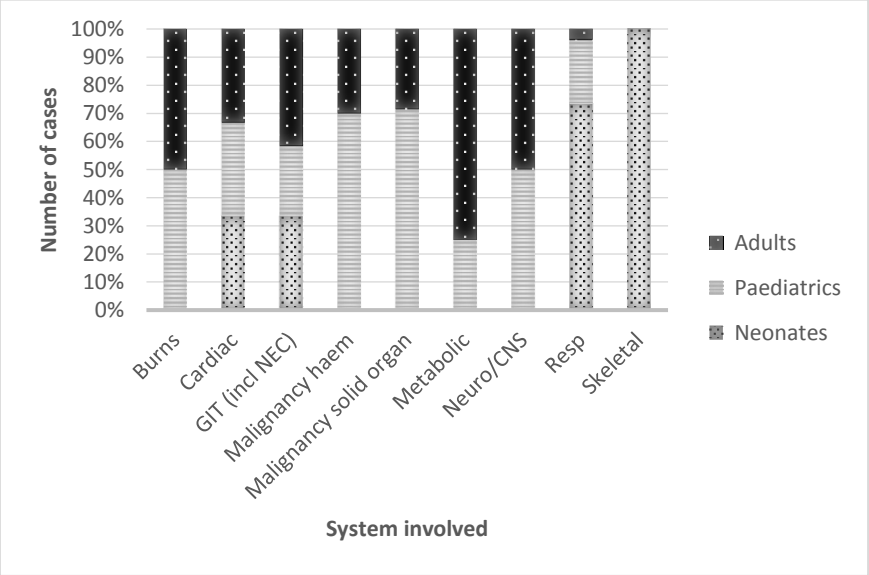

Figure 7. System involved as per clinical diagnosis per age category

Risk factors associated with *C. albicans* versus *Candida* species other than *C. albicans* include the age - neonates, children under 1 year and adults older than 65 years - ward, use of 2 or more antibiotics, use of broad spectrum antibiotics specifically meropenem, aminoglycosides, vancomycin, co-trimoxazole and mechanical ventilation (Table 4). Overall *C. albicans* was the commonest species isolated in HIV exposed or positive patients (18/37, 48.6%) compared to HIV negative patients (51/130, 39.2%) (p = 0.650)

Of the 18 adults with non *C. albicans* candidaemia, 12/18 (66.6%) had GIT involvement and subsequent surgery compared to 6/18 (33.3%) who had involvement of other systems (p=0.155) Significant risk factors in oncology patients included use of central lines or ports. surgical intervention (either insertion or removal of a port) and use of steroids (p < 0.005).

Table 7. Species associated risk factors

	Total	*C. albicans* Number (%)	**Candida* species other than C. *albicans* Number (%)	p value
Age group				
Neonates	71	18 (25,4)	53 (74,6)	**<0.001**
<1 year	93	28 (30,1)	65 (69,9)	
>65 yrs.	4	0 (0)	3 (100,0)	
Ward				
Neonates	71	18 (25,4)	53 (74,6)	**0.001**
Paediatric	56	30 (53,6)	26 (46,4)	
Adults	41	22 (53,7)	19 (46,3)	
Gender	167	69 (41,3)	98(58,7)	0.115
Male	75	26 (34,7)	49(65,3)	
Female	92	43 46,7)	49(53,3)	
Mortality				

	Total	*C. albicans* Number (%)	**Candida* species other than C. *albicans* Number (%)	p value
Overall	59	28 (47,5)	31(52,5)	0.172
Within 7 days	35	16 (45,7)	19(54,3)	0.856
Birth weight < 2,5kg	62	13(21,0)	49(79,0)	0.698
Birth weight < 1,5kg (VLBW)	35	7(20,0)	28(80,0)	
Birth weight < 1kg (ELBW)	13	3(23,1)	9(69,2)	
System involved				0.066
Respiratory	76	25 (32,9)	51(67,1)	
GIT	36	13 (36,1)	23(63,9)	
Any malignancy	24	16 (66,7)	8(33,3)	
Diabetes	8	3 (37,5)	5(62,5)	
HIV exposed	24	8(33,3)	16(66,7)	
Steroids	44	21(47,7)	23(52,3)	0.314
3rd generation Cephalosporin	56	26(46,4)	30(53,6)	0.341
Meropenem	54	14(25,9)	40(74,1)	**0.005**
Aminoglycoside	84	27(32,1)	57(67,9)	**0.015**
Vancomycin	44	12(27,3)	32(72,7)	**0.027**
Chemotherapy	15	9(60,0)	6(40,0)	0.123
2 or more antibiotics	112	41(36,6)	71(63,4)	0.078
Cotrimoxazole	22	18(81,8)	4(18,2)	**<0.001**
Proton pump inhibitor	7	1(14,3)	6(85,7)	0.138
Fluconazole	27	9(33,3)	18(66,7)	0.357
Amphotericin B	15	5(33,3)	10(66,7)	0.510

	Total	*C. albicans* Number (%)	**Candida* species other than C. *albicans* Number (%)	p value
Central Lines	118	46(39,0)	72(61,0)	0.342
Ventilation	81	21(25,9)	60(74,1)	**<0.001**
Abdominal surgery	36	12(33,3)	24(66,7)	0.272

**Candida* species other than *C. albicans* includes *C. glabrata, C. parapsilosis, C. krusei, C. tropicalis* and *C. lusitan*iae

2.1.7 Medicines

Steroid use was similar across all groups with 20/71 (28.16%), 13/55 (23.63%) and 9/41 (21.9%) in neonates. children and adults respectively (p= 0.264). Antibiotic use differed across all groups with 112/167 (67.4%) of patients having received at least 2 broad spectrum drugs prior to the blood culture being taken. Neonates (61/71, 85.9 %), were significantly more likely than children 39/55 (70. 9 %) and adults 12/41 (29. 3%) to have received at least 2 antibiotics (p< 0.001)

Beta lactam use was common throughout all age groups with some patients receiving a single drug simultaneously depending on other concomitant bacterial cultures. Vancomycin, meropenem and aminoglycoside use was highest amongst neonates with 25/71 (35.21%) 10/71 (14.08%) and 49/71 (69.01%) respectively in comparison to the other 2 groups (p <0.001). Co -trimoxazole was used as prophylaxis for *Pneumocystis jirovecii* infection in 18/55 (32.7%) in paediatric oncology compared to adult oncology (4/41, 97.5%) (p = 0.026)

3 Chapter 3

3.1 Discussion

Candidaemia has gained momentum in the past few years as an opportunistic infection predominantly in nosocomial but also in community acquired infections. CHBH has a much higher incidence of candidaemia than most developed countries (Blyth et al., 2009, Bassetti et al., 2011). The differences in candidaemia distribution within this hospital were highlighted in this study*: Candida* species other than *C. albicans* especially *C. parapsilosis* comprising the largest number of isolates was mostly cultured from neonates compared to *C. albicans* in adults in general wards (Bassetti et al., 2015) and paediatric patients. Risk factors associated with the *Candida* species other than *C. albicans* infection were extremes of age, use of invasive devices e.g. mechanical ventilation and use of broad spectrum antibiotics (Cotten et al., 2006). Prolonged hospital stays (> 7 days) noted in all age categories with neonates having the longest (15 days), have been associated with nosocomial infections (Blyth et al., 2009; Menzin et al., 2009; Zaoutis et al., 2005). Additionally, the presence of multiple risk factors for a prolonged period could be drivers for antifungal resistance. In this study, fluconazole resistant *C. parapsilosis* was cultured mostly from neonates and significant risk factors included the neonate category, involvement of the respiratory system, mechanical ventilation, use of a prior antifungal and use of 2 or broader spectrum antibiotics. The overall mortality rate was 35.3% with the was highest number of deaths amongst adults with *C. albicans* infection 15/22 (68.18) in keeping with other studies (Garnacho-Montero et al., 2010; Gokcebay et al. 2016). Significant risk factors associated with in- hospital mortality was use of central lines, urinary catheters, total parenteral nutrition, 2 or more antibiotics, beta lactam - beta lactamase inhibitor, proton pump inhibitor, aminoglycoside and abdominal surgery

3.1.1 Demographics

Similarities in incidence rates to previous studies (Blyth et al., 2009) were noted in neonates having the highest and adults the lowest rates (Blyth et al., 2009). However, the main difference in incidence rates was the higher overall incidence at CHBH, 2.09 per 1000 admissions, almost double compared to similar studies (Blyth et al., 2009, Bassetti et al., 2011). The hospital experiences a high volume of admissions (estimated 150 000 in patients, 500 000 outpatients and 350 accident and emergency and 60 000 maternity patients per year) (URL: https://www.chrishanibaragwanathhospital.co.za/ (accessed 8.22.16). The high incidence may be due to a number of factors. With over 20% HIV positivity rates in the

province and antenatal HIV prevalence almost 30% (Dramowski et al, 2011), a large proportion of patients are immunocompromised with paediatric in -patient mortality reaching 46.1% (Dramowski et al, 2011). Additionally, blood taking practices would usually be dictated by the patient and severity of clinical condition e.g. severely ill patients with suspected sepsis might have blood cultures drawn on admission to an ICU whereas a patient admitted for elective surgery would not. The volume of blood taken is also important as this is problematic in certain patients especially paediatric and neonates because of the low blood volumes submitted for culture. Adherence to infection control policies can be difficult in resource constrained settings due to limitations with staff numbers, high patient volumes, equipment availability e.g. single use items, care of in -situ devices like central lines and feeding practices including milk preparation. This would predispose patients to nosocomial infections and subsequent outbreaks. Concomitant bacterial infections would also drive antibiotic use and increase the selective pressure for resistant bacterial and fungal infections. Inappropriate antifungal prophylaxis, e.g. use of fluconazole when the dominant isolate in a unit is an azole resistant species, would also be a driver for continuing nosocomial transmission. Even though central line care and other infection control measures have not been measured, when coupled with other factors including underlying medical conditions, severe immunosuppression and prematurity, most likely contribute to the high rates observed.

The largest number of patients in this study were children under 1 year of age with more than two thirds being neonates who were premature and of low birth weight (<2500 g). Due to the above factors, most required life- saving interventions like central lines, prolonged mechanical ventilation, steroids and either empiric or prophylactic broad spectrum antibiotics. Prematurity, ICU admission, use of central lines, prior use of broad spectrum antibiotics including the use of more than 2 drugs, GIT surgery and mechanical ventilation have all been shown to be risk factors for neonatal candidaemia (Blyth et al., 2009, Liu et al., 2015, Saiman et al., 2000, Lee et al., 2014, Festekjian A et al., 2012). We confirmed associations with mechanical ventilation and broad spectrum antibiotic use in this study.

The neonatal unit had not only the highest number of cases overall, but also the largest number of admissions (1524 patients) for the study period. Neonates are rapidly colonised mainly in the gastrointestinal and respiratory tracts within 2 weeks after birth (Saiman et al., 2000, Manzoni et al., 2007, Huang et al., 1998). Since fungal colonisation has been shown to be a risk factor for progression to candidaemia, this coupled with the other risk factors for neonates especially prematurity and birth weight, could explain the high burden seen in this

group. Subsequent nosocomial infection with a different species could explain the mixed cultures that were more common amongst this group compared to the adults and paediatrics.

The prolonged length of stay before the first positive blood culture in neonates was 15 days, more than double that noted in other studies (Lee et al., 2013). More than a quarter of these babies also had medical and surgical interventions due to prematurity and low birth weights requiring prolonged stays in the unit, medical devices in situ and ventilation. Factors linked to infection control previously mentioned like overcrowding and difficulties in implementing local infection control policies combined with risk factors for the age group would all have predisposed to nosocomial infections (Vaz et al., 2011). These would need to be investigated and delineated to allow for appropriate application of local infection control protocols.

Even though central line use was high in neonates, it was not statistically significant even though the majority of *Candida* species other than *C. albicans* cultured were *C. parapsilosis*. This species is known to be associated with line colonisation and line sepsis (Vaz et al., 2011). However, in our study, it was not always clear as to the site of the blood culture, i.e. line or peripheral. It is plausible that cultures could reflect either colonisation of a line or the skin, or pseudofungemia from external contamination. This could happen when blood is first used for blood gas analysis before being inoculated into blood culture bottles. Further detailed study of phlebotomy techniques would be required to determine this.

Routine fluconazole prophylaxis for 42 days in preterm or very low birth weight babies had not been instituted at the time of the study. Use has been shown to decrease the incidence of invasive candidiasis but did not have any effect on mortality or increases in azole resistance (Ericson et al., 2014, Che et al., 2016, Cleminson et al., 2015). However, considering the fluconazole resistance rates of > 50% within *C. parapsilosis* isolates (overall fluconazole resistance in all species just under 20%) especially in neonates, the impact of fluconazole prophylaxis would be negligible in that unit. Additionally, the selection pressure from azole use could potentially drive the rates of resistance even higher within this species as well as within those which are inherently resistant e.g. *C. lusitaniae.* This would make choice of empiric antifungals difficult considering the limited antifungal classes available to the public sector.

3.1.2 Mortality

Overall mortality was more than one third with the highest proportion of deaths recorded in the adults admitted to general medical wards (almost half). Although this rate is lower than the 60% previously stated by Kreusch et al in the same setting, it is in keeping with other studies limited to single tertiary centres (Bassetti et al., 2011). The severity of the underlying condition could also have impacted on the choice and timing of treatment as well (Garey et al., 2006, Morrell et al., 2005).

Most of the factors associated with mortality in this study have been previously described (Bassetti et al 2011, Horn et al 2008). All of these are usually interventions in high or intensive care requiring antibiotics as prophylactic or empiric for concomitant infections. Removal or change of devices, if not done timeously would also serve as foci for infection.

The highest species specific crude mortality rates were in adults infected with *C. albicans.* Due to the other clinical risk factors associated with this group, not all cases of death could be directly ascribed to candidaemia. The high *C. albicans* rate noted is in keeping with other studies where *C. parapsilosis* and *C. glabrata* had the lowest mortality rates in adults (Bassetti et al., 2015, Horn et al., 2008). Possible reasons for this could be: treatment of concomitant bacterial or viral infections with broad spectrum antibiotics and of fungaemia coupled with pre -existing risk factors like diabetes could have been a major contributor, continued presence of medical devices like central lines serve as a continuous infective focus in spite of treatment and inappropriate antifungal treatment.

3.1.3 Species

Candida epidemiology in this study is in keeping with other studies overall (Bassetti et al., 2013, Blyth et al., 2009, Bassetti et al., 2015). Within each age cohort, there are some differences. In neonates and paediatrics, *C. parapsilosis* and *C. albicans* were the 2 was most frequently isolated organisms similar other studies (Blyth et al., 2009. Compared to the previous study conducted at CHBH in adults (Kreusch et al., 2013), *C. albicans* fell from 62% to 53%, while *C. parapsilosis* and *C. glabrata* dropped from 27 and 29% to 21% for both organisms in adults.

The general distribution of *Candida* species other than *C. albicans* and *C. albicans* varied amongst the different patient groups and wards. *C. albicans* was still the most common isolate in the general medical wards (Bassetti et al., 2015). In keeping with global trends this study

demonstrated the following: *Candida* species other than *C. albicans* were the commonest species isolated in children under 1 year of age and adults in ICU. This group (*Candida* species other than *C. albicans*) was also associated with risk factors for fungaemia – extremes of age, use of broad spectrum antibiotics and interventions like ventilation.

3.1.4 Clinical diagnoses

Significant associations have been noted between candidaemia, immunosuppression and infecting species (Kreusch et al 2013., Tumbarello et al., 1999, Taunay et al., 1998, Marukutira et al., 2014). HIV positive patients as well as diabetics would already have multiple risk factors for ICI. It is feasible due to the underlying medical conditions, possible multi organ involvement was already established on admission resulting in more septic patients. *C. albicans* and *C. parapsilosis* have been noted to be 2 of the most frequently isolated yeasts in HIV positive patients (Marukutira et al., 2014).

3.1.5 Laboratory

Use of traditional biomarkers like the platelet count have been based on the immunology and interplay between fungi and platelets. However, changes in platelet counts can be difficult to attribute to a particular cause especially in high risk patients with multiple risk factors. In this cohort, the median platelet counts for all groups were below the normal range stipulated by the laboratory with the neonates having the lowest values. For neonatal candidiasis, this is in keeping with other studies where low counts were helpful indicators (Zhao et al., 2014) for diagnosing ICI. Use of 1.3 β –D glucan as a biomarker to aid in diagnosis (Mackay et al., 2011) and prognosticate ICI (Zhao et al., 2014) in different patient populations has been used. Even though this test has been noted to have lower sensitivity in *C. parapsilosis* infections (Mikulska et al., 2016), in this study the specimens from neonates, demonstrated high 1.3 β –D glucan values. This could be indicative of a high burden of infection or a higher organism load if the sample is taken from an infected/colonised line. Further laboratory and clinical investigation would be required to establish this. The converse could apply to oncology patients could have negative 1.3 β –D glucan (blood sampled from the uninfected port) but positive blood cultures taken peripherally.

Isolate identification and susceptibility results guiding treatment were from the CHBH laboratory and polymicrobial blood cultures were identified at NICD. This could have been

due to a number of reasons at CHBH e.g. colony morphology of different species could have been difficult to differentiate and as antifungal susceptibility tests are read at 80% of inhibition, differences could easily have been missed due to subjective variation in reading. The other methods used at the reference laboratory like utilisation of chromogenic media, would have enhanced phenotypic differences enabling easier differentiation of isolates and subsequent isolate specific antifungal susceptibility testing. This could have impacted negatively on choice of antifungal for treatment especially for mixed cultures as the predominant isolate, possibly the azole susceptible one, would have dictated empiric treatment.

3.1.6 Azole susceptibility

The high rates of azole resistant *C. parapsilosis* is cause of concern in this hospital. This was significant, considering that 14.3% of patients on an azole empirically or prophylactically were diagnosed with ICI. High azole resistance impacts choice of empiric and prophylactic antifungal agents in an already resource constrained setting (Govender et al., 2016). Furthermore, nosocomial infection, continuous presence of risk factors and prolonged use of antifungals would also increase the selective pressure and contribute to spread. Molecular techniques could be used to elucidate if strain specific outbreaks are occurring and subsequently aid in choice of empiric treatment (Dizbay et al, 2008).

3.1.7 Strengths and limitations

The retrospective nature of this study meant that not all of the patient files could be located and thus had to be excluded. Even though this represented a degree of bias, overall, the numbers for each group analysed were proportional to the excluded files. The age and prior antifungal category showed significant differences between both groups (age $p < 0.001$; prior antifungal given $p = 0.001$). For neonates, a significantly higher percentage (>50%) of records could not be located compared to a lower % for other age groups

Data regarding concomitant bacterial and other viral infections was not collected and thus correlation with multiple antibiotic use was not done. Patients were not followed up long term to determine morbidity. Information was not always available regarding central line or urinary catheter duration or removal. Patient records did not always indicate if antifungal treatment was prophylactic or empiric or if combination therapy was utilised once ICI was confirmed.

Since hospital data for oncology was not readily available, the incidence in adult oncology could not be calculated.

Blood culture sensitivity for fungal infections is low (Nguyen et al., 2012) and likely worse in neonates and paediatrics due to the limitations on volume, hence multiple cultures taken prior to this might have been negative leading to delays in empirical treatment. On the other hand, improved clinical suspicion, earlier initiation of empiric antifungal therapy and improved use of biomarkers in addition to traditional blood cultures would have contributed to non - culture based diagnoses.

3.2 Conclusion

Overall this study highlights the association of multiple risk factors e.g. invasive devices and use of broad spectrum antibiotics in a hospital setting that contribute to candidaemia.

The differences within units influences antifungal choice for both empiric and prophylactic treatment. Predominance of non-*C. albicans* species combined with the high burden of fluconazole resistant isolates noted in neonates and mixed cultures, use of a broad-spectrum antifungal such as amphotericin B would be indicated until definitive identification and susceptibility testing can be done. Multi- disciplinary educational initiatives targeting infection control and a unit specific antifungal stewardship programme nosocomial infections.

The antifungals that could be used empirically for each group based on this study would include fluconazole for both paediatrics and adults and amphotericin B for neonates. Considering the side effect profile of amphotericin B, use of alternate classes like the echinocandins would need to be explored further in neonates. Considering that complicated *Candida* infection occurs quite commonly in neonates, i.e. meningitis, echinocandin use would also be limited as this drug does not penetrate the cerebrospinal fluid. Thus, the pharmacokinetic and pharmacodynamics properties of the antifungal would need to be taken into account to allow for adequate concentrations at the site of infection.

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5 Appendix

5.1 TRAC SA Lab Case Report Form

Appendix A: TRAC-SA Lab Case Report Form

LAB DETAILS

Lab specimen number: ☐☐☐☐☐☐☐☐☐☐☐☐

Date of specimen collection: d d m m y y

Lab name: _____ Public sector: ☐ or Private sector: ☐

Lab telephone number: ☐☐☐☐☐☐☐☐☐☐

Lab contact person:

_____

PATIENT DETAILS

Hospital number: ☐☐☐☐☐☐☐☐☐☐☐☐

Surname: _____ First name:

_____

Gender: Male: ☐ Female: ☐ Unknown: ☐ Date of birth: d d m m y y

Age: ☐☐☐ Unit: Years: ☐ or Months: ☐ or Days: ☐ Unknown age: ☐

Hospital: _____ Province:

_____

Ward: General: ☐ ICU (General): ☐ Medical ICU: ☐ Surgical/ Trauma ICU: ☐

Paediatric ICU: ☐ High-care: ☐ Burns: ☐ Transplant: ☐ Haematology/ Oncology: ☐

Infectious Diseases: ☐ Other: ☐ Specify: _____ Unknown: ☐

***CANDIDA* SPECIES IDENTIFICATION**

Species name: *C. albicans:* ☐ *C. parapsilosis:* ☐ *C. glabrata:* ☐ *C. krusei:* ☐

C. tropicalis: ☐ *Candida* species not identified: ☐ Other species: _____

Identification method: API 20C: ☐ API 32C: ☐ Auxacolor: ☐ CHROMagar-Candida: ☐

Microscan: ☐ Vitek: ☐ Germ tube test only: ☐ Other: ☐ Specify: _____

***CANDIDA* SUSCEPTIBILITY TESTING** Not done for this isolate: ☐

Fluconazole: Zone size: ___mm or MIC: ___mg/L Test method: _____ Not done: ☐

Amphotericin B: Zone size: ___mm or MIC: ___mg/L Test method: _____ Not done: ☐

Voriconazole: Zone size: ___mm or MIC: ___mg/L Test method: _____ Not done: ☐

Other drug: _____ Zone size: ___mm or MIC: ___mg/L Test method: _____

5.2 TRAC SA Clinical data

Appendix B: TRAC-SA Telephone Interview Form

COMPLETE THIS FORM WHEN THE POSITIVE BLOOD CULTURE RESULT IS COMMUNICATED TO THE CLINICIAN

1. Please confirm the type of hospital ward to which the patient was admitted when the positive blood culture was collected:
   General: ☐ ICU (General): ☐ Medical ICU: ☐ Surgical/ Trauma ICU: ☐
   Paediatric ICU: ☐ High-care: ☐ Burns: ☐ Transplant: ☐ Haematology/
   Oncology: ☐ Infectious Diseases: ☐ Other: ☐ Specify: _____
2. Was the patient on antifungal treatment at the time that the positive blood culture was collected?
   ☐ Yes
   ☐ No
   ☐ Unknown
3. If yes to Q5, please specify antifungal drug:_____
4. Has an antifungal drug been recommended, based on the positive blood culture?
   ☐ Yes
   ☐ No
   ☐ Unknown
5. If yes to Q7, please specify which antifungal drug has been recommended:_____

5.3 Additional clinical data

Admission (1st time)								
Date			dd/mm/yy		Duration			
Ward	Adult			Paeds				
Transfer from a facility								
Date				Specify				
Outcome								
Discharge				Death		Unknown		
Transfer				Specify facility				
Paediatrics (neonates <30 days)								
Prenatal history (Mother)								
Medical history					Specify			
HIV		Current CD		Perinatal ARV's			Duration	
Other medication								
Mode of delivery and reason if C/S								
Patient								
Gestational Age at birth			Apgar scores		5 minutes		10 minutes	
Birth weight								
Reason for admission			Neonatal sepsis (no obvious focus)					
System inv	Respiratory	Cardiac	Abdominal	CNS	Other (spe	Complication		
Interventions required		Ventilation	Central line	TPN	Surgery	Foley's catl	Other (specify)	
Medicatior	PPI	Steroids	Antibiotics/antifungals					
Drug								
Dose/Method admin								
Duration								
Adults/Paediatrics (>30 days)								
Diagnosis		Sepsis without obvious focus						
System inv	Respiratory	Cardiac	Abdominal	CNS	Haematolo	Trauma	Other (specify)	
Med histor	Diabetes	HPT	Steroid use	Immunosu	Chemother	Other (specify)		
HIV	Status	Current CD	Viral load	ARV's (star	OI (specify)	Prophylaxis		
Interventio	Ventilation	Central line	TPN	Surgery	Foley's cath	Other (specify)		
Medicatior	PPI	Steroids	Antibiotics/antifungals					
Drug								
Dose								
Duration								
Investigation date								
	CRP	WCC	Beta D gluc	Platelets	Blood cultu	Isolate	MIC Flucon	Vori
Specify site	for BC							

5.4 Ethics clearance

INVESTIGATORS	Dr Sharona Seetharam.
DEPARTMENT	Clinical Micorbiology and Infectious Diseases
DATE CONSIDERED	29/06/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 29/06/2012 **CHAIRPERSON** .....

(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr Nelesh Govender

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

5.5 Turnitin Report