Neonatal Candidaemia at Universitas Academic Hospital, Bloemfontein, South Africa

Submitted in fulfilment of the requirements in respect of the Master's Degree MMed in the Department of medical microbiology in the Faculty of health sciences at the University of the Free State.

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Declaration of authorship:

I, Dr L.S. Mnqokoyi, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification MMed in the Department of medical microbiology in the Faculty of health sciences at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

ABSTRACT

BACKGROUND: Candida remains the most common cause of morbidity and mortality during the neonatal period. Knowledge of epidemiology of candida species and their antifungal profile is important to guide empiric therapy. The aim of the study was to characterise and evaluate the antifungal susceptibility of candida species causing neonatal bloodstream infections at Universitas Hospital. Vitek 2 automated system is used for yeast identification and antifungal susceptibility. To determine the accuracy of the results generated, the antifungal susceptibility results were compared to those generated at the reference laboratory in Johannesburg.

METHODS: A retrospective, laboratory based study was conducted over a one year period. Isolates from neonates with candidaemia were identified to species-level and antifungal susceptibility testing was performed. Vitek 2 yeast cards were used for identification and antifungal susceptibility testing. Antifungal susceptibility to fluconazole, amphotericin B, and caspofungin obtained in our laboratory were compared to those obtained at the reference laboratory. At the reference laboratory, broth microdilution susceptibility testing was performed. Categorical agreement was calculated for susceptibility results.

RESULTS: Overall a total of 45 candida species were detected. Non- albicans candida accounted for 73% of neonatal candidaemia, with *Candida parapsilosis* being the most prevalent (60%). *Candida albicans* was the second most common isolate (27%).

Overall 23 (85 %) of the *Candida parapsilosis* isolates tested were resistant to fluconazole. When Vitek 2 antifungal susceptibility was compared to broth microdilution performed at the reference laboratory, CA was 100% for amphotericin B and caspofungin. However, for

Candida parapsilosis, the CA for fluconazole was 50% with 4 (18%) major errors and 7 (32%) minor errors.

CONCLUSIONS:

Candida parapsilosis is the major cause of candidaemia amongst the neonate in this study. Amphotericin B is currently the appropriate empirical drug of choice for treatment of this infection.

Key words: Candidemia, Neonatal septicaemia, antifungal susceptibility testing

Background

Candidaemia remains a major cause of mortality and morbidity worldwide[1-3]. Neonatal candidemia ranks as the third leading cause of bloodstream infection (BSI) and accounts for nine to 13% of BSI in developed countries[1-3]. In low income countries, particularly in Sub-Saharan African settings, the epidemiology of candidaemia is not well described[4]. In a Tanzanian prospective cohort of children less than seven years, candidaemia accounted for approximately nine percent of all BSI[4].

The spectrum and burden of candida BSI fluctuates between geographic regions and within neonatal units[5-8]. Although *Candida albicans* is implicated in most cases of candidaemia, several studies have reported a rising number of infections caused by non-albicans candida (NAC) species [5-9.] It is therefore necessary to monitor candida species isolated in any specific setting, as well as its antifungal activity as empiric treatment is modified based on the knowledge of the local epidemiological data.

In South Africa, a prospective multicentre laboratory-based survey of candidaemia that describes the epidemiology of candidaemia and its antifungal profile in both public and private sector hospitals has been published for 2009 to 2010[9]. A study describing the epidemiology of candidaemia in both children and adults in Bloemfontein was published more than a decade ago[10].

The aim of the study was to characterise and evaluate the antifungal susceptibility of candida species causing neonatal bloodstream infections at Universitas Hospital as well as to determine the accuracy of antifungal susceptibility testing performed by the Vitek 2 automated assay by comparing results to microbroth dilution performed at the reference method.

METHODS

This was a retrospective, laboratory based study conducted between January 2016 and December 2016 at the Universitas Diagnostic Microbiology Laboratory, National Health Laboratory Services, University of the Free State, Bloemfontein. Isolates from neonates with candidaemia were identified and their antimicrobial profile determined.

A neonate was considered to be any patient admitted within 28 days of life or those born and have never been discharged. Neonatal candidaemia was defined as a laboratory confirmed BSI where one or more blood culture specimen yielded any candida species. Repeat isolates of candida species within seven days of the original isolate were considered to represent a single episode of infection. An audit of laboratory information system was carried out to ensure completeness of reporting.

Preliminary identification was based on colonial morphology on sabouraud dextrose agar and germ tube test. Confirmation identification was done with carbohydrate fermentation and assimilation test using the Vitek 2 (bioM´erieux Inc, Marcy l'Etoile, France) automated assay. The API32C Aux Yeast (bioM´erieux Inc, Marcy l'Etoile, France) was used for organisms that were not identified by the automated assay. Isolates were submitted to the National Institute of Communicable Diseases (NICD) as part of the national surveillance program. At the NICD reference laboratory, identification and antifungal susceptibility testing was repeated to confirm the results.

Antifungal susceptibility testing (AST) was performed using the Vitek 2 automated assay. Minimum inhibitory concentrations (MIC) for fluconazole, caspofungin and amphotericin B were determined. Results obtained with the Vitek 2 assay were compared to those obtained at the reference laboratory. At NICD, MIC were determined using pre-prepared dried Sensititre

YeastOne microbroth dilution panels containing Alamar Blue (Thermo Fisher Scientific, Cleveland, Ohio, USA). Minimum inhibitory concentration results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI)[13.]

Categorical agreement (CA) was calculated. Very major errors (VMEs) were identified when the reference MIC indicated a resistant result and the Vitek 2 assay was susceptible. Major errors (MEs) were identified when an isolate was classified as resistant by the Vitek 2 assay and susceptible by the YeastOne method. Minor errors were identified when the isolate was classified as susceptible, dose dependent by one system and either susceptible or resistant by the other system.

Epidemiological cut off (ECOFF) values were used for amphotericin B as there is no interpretative criteria for candida species. Isolates with an amphotericin B MIC of <1 mg/L were considered to be susceptible to this agent.

STATISTICAL ANALYSIS

An excel spreadsheet was used to capture data. Descriptive analysis was done by the Department of Biostatistics, UFS using SAS Version 9.4. Categorical data was summarised by percentages and frequencies.

RESULTS

Candida species identification and susceptibility at Universitas Microbiology Laboratory

During the study period, a total of 45 candida species were detected at Universitas hospital. *Candida albicans* constituted 27 % of all candida species isolated while the majority were NAC. *Candida parapsilosis* (60%) was the most commonly isolated organism (Fig 1). *Candida lusitaniae and Candida famata*, both uncommon cause of neonatal candida infections, accounted for four percent of candidemia in the study.

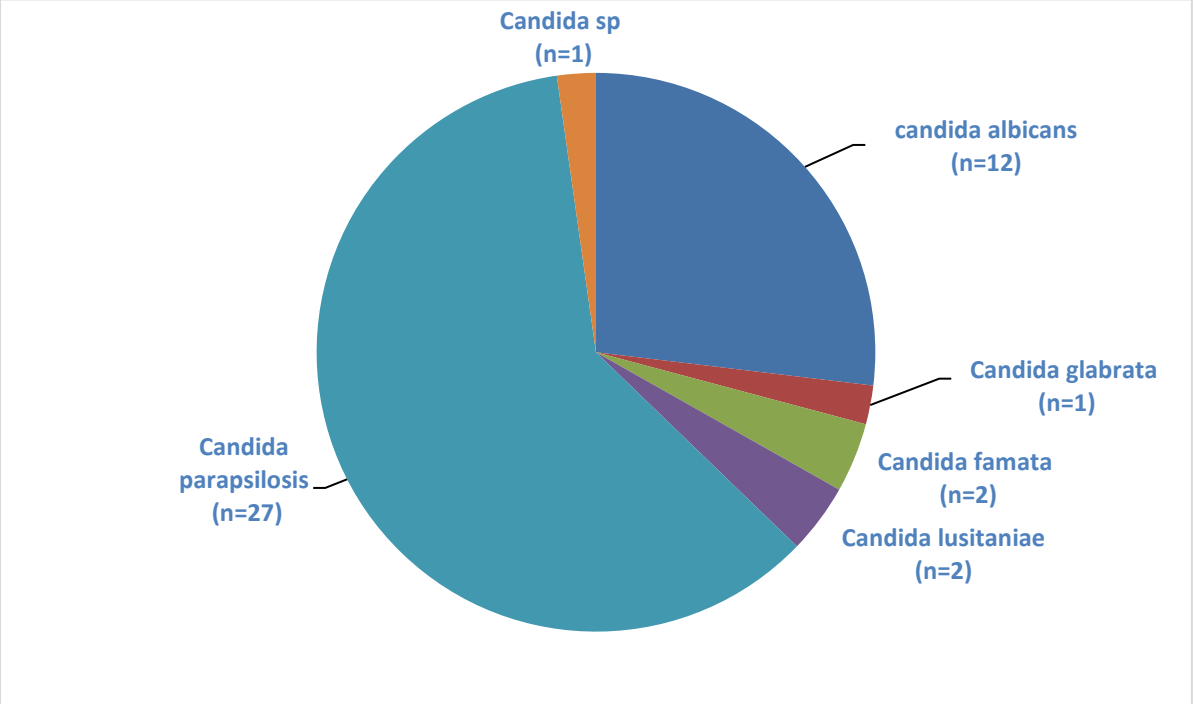

Figure 1: Characterisation of candida species isolates from neonatal blood cultures (n=45)

Regarding AST, all *Candida albicans* isolates were susceptible to fluconazole. Overall 23/27 (85%) of the *Candida parapsilosis* isolates were resistant to fluconazole (Table 1). There are no fluconazole breakpoints for *Candida lusitaniae* but the MIC values were below 0.5 µg/ml and considered susceptible.

There are no interpretative breakpoints for amphotericin B for all candida species. All candida isolates including *C. albicans, C. glabrata, C. parapsilosis and C. famata* had MIC values less than 1 µg/ml. These were considered susceptible based on the ECOFF values that have been established by CLSI[14]. All candida species for which echinocandins were tested were found to be susceptible (Table 1).

Table 1: **Resistance results of candida species as performed at Universitas microbiology laboratory**

Candida species	**Number**	**Fluconazole resistance**	**Caspofungin resistance**	**Amphotericin B resistance**
C. albicans	12	0	0	0
C. parapsilosis	27	23	0	0
C. lusitaniae	2	0	0	0
C. glabrata	1	0	0	0
C. famata	2	NBP	NBP	NBP
C. species	1	ND	ND	ND

NBP – no CLSI breakpoints, ND – not done

Comparison with the reference laboratory

Comparing species identification with the reference laboratory, species level identification was identical in majority of the cases except for two *Candida famata* isolates which were identified as *Candida parapsilosis* at NICD. One candida species that could not be identified at Universitas Microbiology laboratory to species level was also identified as *Candida parapsilosis* at NICD, further increasing the prevalence of *Candida parapsilosis* bacteraemia.

When comparing antifungal susceptibility results between the Vitek 2 assay used at Universitas Microbiology laboratory and YeastOne method used at the reference laboratory, we excluded all the isolates for which antifungal susceptibilities were not performed with the Vitek 2 assay and those with discordant species identification (Table 2). A total of 29 isolates tested were eligible for comparison.

Table 2: Candida isolates excluded from the analysis (n = 16)

Organism(n)	Reason(s)
C. parapsilosis (5)	Alternative methods performed for identification and susceptibility
C. albicans (6)	Alternative methods performed for identification and susceptibility
C. lusitanie (2)	There are no interpretative breakpoints
C. famata (2)	Discordant species identification. There are no interpretative breakpoints
Candida species (1)	Could not be identified to species level at the Universitas laboratory

All *C. albicans* (6/6) and *C. glabrata* (1/1) isolates were reported as susceptible to fluconazole by the Vitek 2 assay and YeastOne method with a CA of 100%. A significant number of *C. parapsilosis* were reported as resistant to fluconazole by the Vitek 2 assay compared to YeastOne method. Major errors were recorded in four (18%) isolates and seven (32%) isolates had minor errors (Table 3). All of the candida isolates tested by both methods were found to be susceptible to caspofungin and amphotericin B with no errors detected. There were no very major errors for all tested antifungals.

Table 3: Comparison of fluconazole, caspofungin and amphotericin B susceptibility results between Vitek 2 assay (Universitas lab) and YeastOne method (NICD) (n=29).

	CA %	**mE %**	**ME %**	**VME %**	**CA %**	**mE %**	**ME %**	**VME %**	**CA %**	**mE %**	**ME %**	**VME %**
***C. albicans* (6)**	100	0	0	0	100	0	0	0	100	0	0	0
***C. glabrata* (1)**	100	0	0	0	100	0	0	0	100	0	0	0
***C. parapsilosis* (22)**	50	32	18	0	100	0	0	0	100	0	0	0

Note: CA – categorical agreement; me – Minor Error; ME – Major Error; VME – Very Major Error, ( ) - no of isolates tested by both AST methods

DISCUSSION

Neonatal candidaemia has a high attributable mortality requiring appropriate antifungal therapy to be commenced early in order to improve patient outcome[15-17]. Neonates suspected of invasive candidiasis are started on empiric antifungal therapy while awaiting culture results[17]. Current data on neonatal fungal isolates and their antifungal susceptibility pattern is required to guide empiric therapy. At Universitas Academic Hospital, fluconazole was the empiric drug of choice prior to the study, but was then changed to amphotericin B based on surveillance data generated from the laboratory in 2016.

In the current study, the dominant yeast pathogen was *Candida parapsilosis*. This finding is consistent with other studies conducted in South Africa. A single-unit study undertaken at Johannesburg hospital between 2007 and 2011, which focused on neonatal BSIs showed that *C. parapsilosis* (54.2%) was the most common organism isolated followed by *C. albicans* (27.1%) [7]. A national survey of candidaemia undertaken from 2009 to 2010, documented *C. parapsilosis* as an emerging and important cause of candidaemia in South Africa both in adults and children[9]. In the neonatal group, *C. parapsilosis* was the most common cause of candidemia[9].

In contrast, *C. albicans* was the most frequently isolated species (69%) at Tygerberg Hospital in Western Cape Province in 2008 followed by *C. glabrata* (10%), *C. parapsilosis* (10%) and other species (11%) [11]. This study included both adults and children. In addition, a retrospective survey conducted in Bloemfontein in the year 1989 showed *C. albicans* (42%), followed by *C. tropicalis* (26%) and *C. parapsilosis* (20%) were the species most frequently isolated[10]. These studies underscore the importance of continuously or periodically monitoring trends of candida species as it differs between geographic areas and change with time.

Majority of the *C. parapsilosis* isolates (85%) were resistant to fluconazole. This was of great concern because fluconazole was amongst the first line antifungal agent used for empirical treatment of candida BSI in Universitas neonatal unit. Although less virulent than *C. albicans*, *C. parapsilosis* have proven more challenging to manage because of increasing antifungal resistance and propensity to form biofilms[12]. In other regions of the world, for instance in Western China, most *C. parapsilosis* remain susceptible to fluconazole[17]. However, our findings were in keeping with the Johannesburg hospital study, which demonstrated that half of the neonatal cases caused by *C. parapsilosis* were due to a fluconazole-resistant strains[8].

There were two *C. parapsilosis* isolates that were misidentified as *C. famata* by the Vitek 2 assay. There are some reports documenting misidentification of *C. parapsilosis* and *C. guilliermondii* as *C. famata* when using Vitek 2 assay[19,20]. This is largely attributed to similar biochemical and phenotypic characteristics of these yeasts. This finding highlight the importance confirming the identification when rare yeast species are identified with automated identification systems.

All study isolates tested for caspofungin susceptibility for which there are CLSI-defined species-specific breakpoints, were susceptible. Caspofungin susceptibility was tested even though testing has been reported to be unreliable due to batch to batch variability [9,18]. Anidulafungin and micafungin are considered as surrogate markers for echinocandin but are not available in public sector hence caspofungin was used [9]. There are few data published on the use of echinocandins in neonates. There are also concerns with echinocandins use because achievable concentrations in some tissues including central nervous system, retina and kidney are low and neonatal candida infections can invade virtually all tissues[21].

Isolates for which antifungal susceptibilities were not performed with the Vitek 2 assay and those with discordant species identification were excluded from the analysis and this reduced the number of isolates eligible for comparison.

There was good CA between the two methods for *C. albicans* for all three antifungals, however for *C. parapsilosis,* four (18%) MEs and seven (32%) minor errors were observed for fluconazole. Categorical agreement between the two methods for fluconazole was 50%. Most of the isolates that were reported as resistant by the Vitek 2 assay were categorised as susceptible dose dependent by YeastOne method. This is an important finding as fluconazole may still be the preferred drug for some patients over amphotericin B, due to high oral bioavailability and low incidence of side-effects.

BioMerieux has since introduced a new and improved yeast antifungal susceptibility card and a software has been updated to expand the database. . This will strengthen the correlation between the routine diagnostic method and those used at the reference laboratory thus improving diagnostic accuracy.

The main limitation of the study was the retrospective design and the small sample size. A prospective study with a large sample size is recommended. In addition, essential agreement between the two methods of antifungal susceptibility could not be calculated as the Vitek 2 assay gives a range not the absolute MIC.

CONCLUSION

Candida parapsilosis is the major cause of candidaemia amongst the neonate in this study. Amphotericin B is currently the appropriate empirical drug of choice for treatment of this infection.

Abbreviations

AST-Antifungal susceptibility testing, BSI-Bloodstream infection, CA-Categorical agreement, CLSI- Clinical and Laboratory Standard Institute, ECOFF - Epidemiological cut off, MEs – Major errors, NAC-Non-albicans candida ,VMEs - Very major errors, UFS – University of Free State

Declarations

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Availability of data and material

Data will be made available on requests from the corresponding author

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Authors' contributions

LM was responsible for the study design, analysis and writing of the publication. MM supervised the study and revised the manuscript critically

Ethics approval and consent to participate

This study was approved by the Bioethics Committee of the University of the Free State (HSREC 105/2016) and Bioethical Committee of Frere State Department of Health (FS2016RP34977). Isolates were not linked to individual patients; therefore, no consent was required from the patients

Consent for publication

Authors have reviewed and approved the manuscript for publication.

Competing interests

Authors declare that they have no competing interests

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