A case control study of candidemia in very low birthweight infants in a tertiary hospital in Johannesburg.

Student: Dr Carol Jacobeth Malunga

Student no: 1261378

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Supervisor: Prof Daynia Ballot (PhD Med)

Principal neonatal/ paediatrics Specialist

Department of Paediatrics and Child Health CMJAH

Co-supervisor: Dr Trusha Nana

Pathologist: Microbiology

Clinical Microbiology and Infectious Diseases, NHLS, WITS

A research report submitted to Faculty of Health Sciences WITS, as a requirement for completion of Masters of Medicine; Paediatrics. Johannesburg, South Africa 2018.

Table of contents:

Dedication.

To my mother Aus. Shonono and my children Lesego, Thato, Lebogang, Orifha and grandchildren Kgosi and Kganya, thank you for your unwavering support and encouragement.

Declaration.

I Carol Jacobeth Malunga declare that this research report is my own work. I am submitting it for the degree of Masters of Medicine in Paediatrics, University of the Witwatersrand, Johannesburg, South Africa. The work has not been submitted before for publication or degree purposes at this or other universities.

Signed: Dr Carol Jacobeth Malunga.

Date: 27 May 2019

Place: Nelspruit, Mpumalanga.

Acknowledgements.

I am grateful to Prof Ballot and Dr Nana my two supervisors for their guidance and support from inception to completion of this research project. Rosella for assisting with accessing data and ensuring that correct data is accessed and Glory for teaching me how to interpret statistical results.

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Abstract.

Background.

Candidemia is a significant cause of morbidity and mortality in infants. The mortality rate ranges between 21% and 76%. Non-albicans candida (NAC) is increasing in incidence and resistance to azoles.

Very low birth weight (VLBW) infants have numerous risk factors which predispose them as a group to invasive candidemia.

Methods.

A retrospective case control study of candidemia in VLBW infants admitted to the neonatal unit at Charlotte Maxeke Johannesburg Hospital (CMJAH) between 01 January 2015 to 31 December 2017 was undertaken.

Clinical and demographic characteristics of VLBW infants who developed candidemia, commonest Candida species, antifungal susceptibility profiles and outcomes defined as death were identified**.**

71 infants with confirmed positive blood cultures for candidemia from the NHLS database were selected and each case was allocated 3 controls; the final sample comprised 284 infants.

Results.

Bacterial sepsis, chronic lung disease (CLD), necrotising enterocolitis (NEC) and NEC surgery, other surgery, anaemia and ventilation, all showed a strong association with development of candidemia in the infants.

The most common isolate was *Candida parapsilosis* (59.1%), followed by *Candida albicans* (30.9%). The cases of candidemia overall and NAC isolates increased over the study years. Resistance to azoles by NAC was demonstrated.

Mortality was 31.2% and 28.2% in controls and cases respectively. The difference in death between the two groups was not statistically significant.

Conclusions.

The study demonstrated a predominance of NAC isolates, increasing rate of candidemia and increased resistance to azoles. Stricter infection control measures and medical intervention strategies should be implemented.

Introduction.

The commonest cause of invasive fungal infections in infants is *Candida* species. Candidemia is associated with significant morbidity and mortality in infants . The mortality rate in infants with candidemia ranges between 21% and 76% [(2)] . Ballot et al in their review of neonatal fungal bloodstream infections (BSI) at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) for the period January 2007 to December 2011 reported a mortality rate of 45.8% [(3)]. Candidemia is responsible for 9-13% of BSI in infants[(3)]. A high index of suspicion for candidemia needs to be maintained in infants of very low birth weight (VLBW), low gestation, CLD, anaemia, surgery, necrotising enterocolitis (NEC) , bacterial sepsis , hypothermia and low blood glucose in whom blood cultures remain negative [(3)]. Symptoms and signs of candidemia are non-specific and blood cultures have a suboptimal sensitivity for detection of candidemia. The burden of disease, poor outcomes and cost of medical treatment in infants with candidemia is significant and necessitates the implementation of prevention strategies.

Infants at most risk for fungal BSI are VLBW and extremely low birth weight (ELBW) infants, infants born at less than 30 weeks gestation age and those with surgical problems [(4)]. The risk of sepsis is inversely proportional to birth weight and gestation age[(5)]. VLBW infants are prone to numerous medical complications which require invasive treatment and a significant number of the treatments can increase the risk for candidemia.

Infants with candidemia present with non-specific clinical signs. Sudden clinical deterioration; such as elevated or low blood glucose levels, unexplained abdominal distention, intolerance to feeds (large gastric aspirates or aspirates with abnormal discoloration), hypothermia, sudden increased ventilation or oxygen requirements, lethargy, platelet levels dropping and circulatory insufficiency require urgent treatment [(3, 4)].

Appropriate choice of antifungal agent and timely implementation of therapy are significant with respect to patient outcomes. A delay of more than 24 hours in administration of appropriate therapy has been shown to independently increase the patient's probability of demising by two fold [6]. The selection of appropriate empiric therapy must take into account the local epidemiology. In settings with a high prevalence of non-albicans and azole-resistant Candida species, the use of fluconazole is not suitable for empiric therapy.

Management guidelines must be guided by local current epidemiological data. This study therefore aimed to review candidemia in VLBW infants at CMJAH South Africa, the associated risk factors, the commonest fungal organism isolated and the antifungal susceptibility patterns.

Objectives

- To describe the characteristics (gender, birth weight, Hyaline membrane disease (HMD), chronic lung disease, anaemia, maternal HIV, antenatal steroids, antenatal care, NEC, NEC surgery, ventilation and bacterial sepsis) of neonates with fungal BSI and those without fungal BSI between 01 January 2015 and 31 December 2017 and to compare the two groups.
- To describe the epidemiology of candidemia, including the antifungal susceptibility patterns of isolated fungal pathogens.
- To describe the risk factors associated with fungal BSI.
- To determine outcomes of neonates with candidemia.

Methods.

This was a retrospective case control study of VLBW infants admitted to the neonatal unit at CMJAH between 01 January 2015 to 31 December 2017.

A review of candidemia in VLBW infants in CMJAH South Africa was taken.

Data used was obtained from Research Electronic Data Capture (REDCAP) hosted by the University of the Witwatersrand [7]. The VLBW infants identified with

candidemia were classified as cases. The next three VLBW infants, without candidemia, admitted to the unit were classified as controls.

Culture results (organism identity and antifungal sensitivity) were obtained from the National Health Laboratory Services data base (Corporate Data Warehouse). Those infants with suspected candidemia who were found to have negative cultures were excluded from the study.

During the study period Amphotericin B was used as empiric therapy for treatment of suspected candidemia. Treatment was revised based on microbiology results.

The BacT/AlerT® (BioMerieux, France) blood culture system was used. Yeasts were identified using Vitek® MS, Vitek® 2 or API® 20C AUX (BioMerieux). Antifungal susceptibility testing was performed using ETESTs® or Vitek® 2 (BioMerieux) and interpreted using Clinical and Laboratory Standards Institute guidelines (CLSI M27-A3)[8].

The participants were categorised according to their weight, those <1000g and >1000g and gestational ages <30 and >30 weeks. The cut off for weight was 1500g and gestational age <36 weeks.

Definitions.

Chronic lung disease (CLD) was defined as supplemental oxygen at 28 days of age.

Necrotising enterocolitis (NEC) was defined as modified Bell's stage 2 and 3[9].

Bacterial sepsis was defined as culture proven sepsis at any age.

Statistical analysis

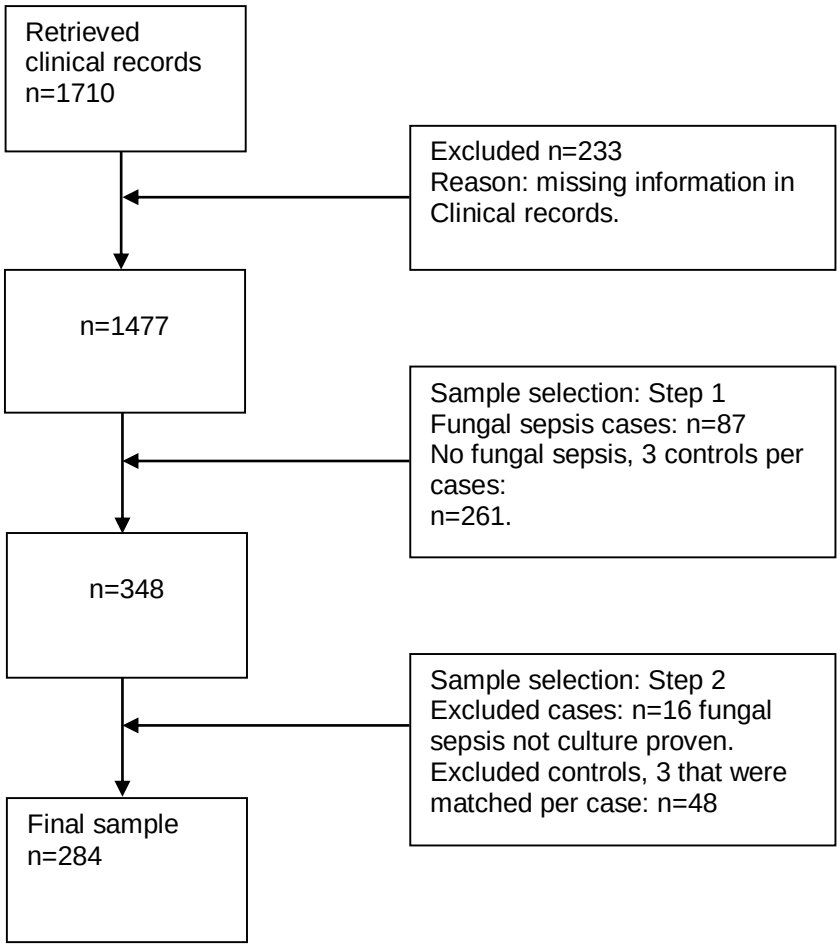
IBM SPSS statistics version 25 was used for statistical analysis. Categorical data were described using frequencies and percentages. Cases (with candidemia) were compared with controls (without candidemia). Chi- square analysis was used to compare categorical data. A p-value <0.05 was considered statistically significant.

Ethics.

The protocol was submitted for approval to the Human Research Ethics Committee (medical) of the university of the Witwatersrand. Permission to conduct the study was granted from the department of Paediatrics and Child Health, as well as from the CMJAH chief executive officer.

Results:

Flow diagram showing selection of study participants.



The majority of the patients were females (54.6%). Most infants were VLBW (65.1%) and below 30 weeks gestation (64.0%). The overall mortality rate was 30.6%. There was no association with candidemia for gender, birthweight, gestational age or mortality in the current study. Chronic lung disease (CLD), anaemia, NEC, the need for ventilator support, surgery and bacterial sepsis were all significantly associated with candidemia. (See Table 1).

Table 1: Patient characteristics in VLBW infants with and without candidemia

Characteristics	Total(n)%284	No FBSI(n)%213	FBSI (n)%71	*P*-value
Gender Male	129(45.4)	99(46.5)	30(42.3)	0.583
Gestational age <30 weeks	181(64.0)	132(62.0)	49(69.0)	0.322
Birth weight <1000g	97(34.1)	75(35.2)	22(31.0)	0.536
HMD CLD Anaemia	206(72.5) 32(11.3) 85(29.9)	153(71.8) 16(7.5) 54(25.4)	53(75.0) 16(22.5) 31(43.7)	0.643 0.002 0.005
Maternal HIV	93(32.7)	65(30.5)	28(39.4)	0.205
Antenatal steroids	118(41.5)	84(39.4)	34(47.9)	0.404
Antenatal care	202(71.1)	151(70.8)	51(71.8)	0.761
NEC	41(14.4)	24(11.3)	17(23.9)	0.012
NEC surgery	32(11.3)	19(8.9)	13(18.3)	0.049
Other surgery	29(10.2)	10(4.7)	19(26.8)	<0.001
Ventilated NCPAP Conventional	206(72.5) 99(34.9)	142(66.7) 55(25.8)	64(90.1) 44(62.0)	0.001 <0.001
Bacterial sepsis:	118(41.5)	62(29.1)	56(78.9)	<0.001
Demised	87(30.6)	67(31.5)	20(28.2)	0.324

HMD: hyaline membrane disease; CLD: chronic lung disease; NEC: necrotising enterocolitis; NCPAP: nasal continuous positive pressure ventilation. Other surgery (arthrotomy, arthroplasty, laparotomy for various indications).

Microbiology.

Table 2

Organisms isolated and antimicrobial sensitivity.

Candida species isolated	Total	Frequency	Azole susceptible	Total % resistant
C. parapsilosis	*42	59.2%	20/34	41.2%
C.albicans	22	31.0	22	0
Other Candida species	4	5.6	-	-
C. glabrata	3	4.2	2	-

*Data missing for *C. parapsilosis* microbiology results, only 34 out of 42 reported.

*No CLSI breakpoint for *C. glabrata* and voriconazole.

Figure 1 shows cases of the different candida isolates during the study period. *C. parapsilosis* showed an increase cases in all the years. The increase over the years of cases of candida isolates was not statistically significant ($P = 0.835$). The incidence of candidemia in this VLBW study population was calculated as total number of candidemia cases divided by the number of total admissions of VLBW infants (18/475, 23/487, 30/515 for 2015, 2016 and 2017 respectively) to get percentage total per annum.

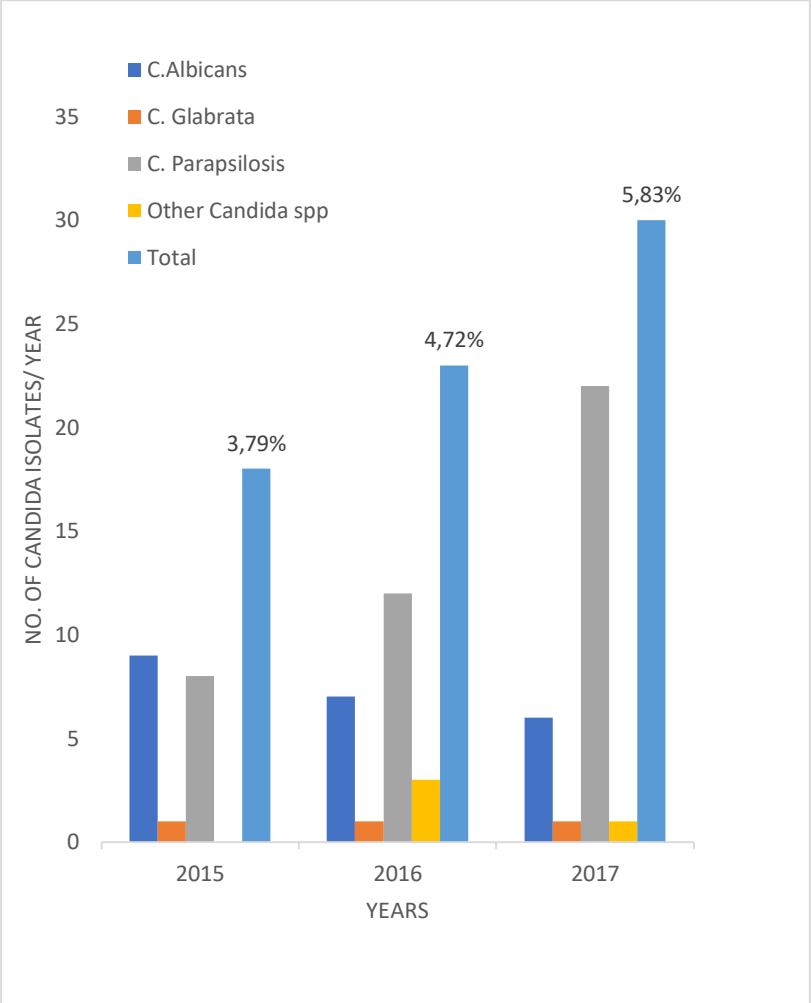


FIG. 1. Number of Candida species (isolates per year). The percentages indicate the percentage of VLWB infants with candidemia per year of the study.

Discussion.

The study found an increase in the cases of candidemia in VLBW infants at CMJAH. There was an increase in the cases of candidemia during the study period from 3.79% in 2015 to 5.83% in 2017, an increase of almost 50% over a three-year period. This was in keeping with a previous report from the same study unit which demonstrated an increase in candidemia over a five year period [4]. Lovero et al showed an increase in the incidence of non-albicans Candida (NAC) with particular reference to *C.parapsilosis*[10]. The study demonstrated an increase in the cases of *C. parapsilosis* from 2015 to 2017 and the results were statistically significant with a p-value of <0.001.

The risk factors significantly associated with candidemia in VLBW infants in the present study were similar to those reported in western China and India [11-13]. The association between candidemia and NEC, mechanical ventilation and surgery reported in the current study are comparable with other studies evaluating candidemia in infants [4, 14-16] . The association between NCPAP and candidemia described above had not been shown previously. The plausible explanation would be poor adherence to infection control measures. When the tubing and humidifiers are contaminated by the hands of the healthcare personnel possibly colonised with fungal organisms it can lead to contamination and spread of fungal infections [17]. Therefore, adhering to strict infection control measure is essential. CLD has been found to be a risk factor for candidemia in VLBW by the present study ,Won et al, demonstrated a similar association[18].Low gestation (<30 weeks) and ELBW (<1000g) were not statistically significant risk factors in this study. However risk factors that are found to be common to VLBW infants are seen in this study and have shown a strong association with candidemia [12, 17, 19].

Prior bacterial sepsis was a common risk factor in patients presenting with candidemia. Broad spectrum antibiotics are used for treatment of bacterial sepsis. This is associated with a selective pressure for Candida species [1, 20]. The study showed bacterial sepsis to have a significant association with candidemia in VLBW infants, and is consistent with results in similar studies [1, 4, 15]. At the time of the study the empiric antibiotics for late onset bacterial sepsis were meropenem and vancomycin. Fu et al demonstrated a strong association between carbapenem use and development of candidemia [13].

Candida species is a third leading cause of late onset sepsis recovered in blood culture isolates and leading cause of fungal infection in infants, in particular VLBW [6, 10, 14, 21]. The present study has shown an increase in the incidence of candidemia. A decline in the number of *C. albicans* blood culture isolates is seen this study, with *C. parapsilosis* blood culture isolates increasing annually and surpassing *C. albicans*. Studies from Brazil, Iran, Uganda and Netherlands have demonstrated an increase in NAC however, *C.parapsilosis* was the second isolate to *C.albicans* [12, 15, 21, 22]. Fu

et al showed *C. glabrata* as the second common NAC isolate in a recent study done at a tertiary hospital in western China[14].

Hospitals in Italy and in the USA demonstrated a strong association between *C. parapsilosis* and the use of total parenteral nutrition (TPN) and adherence to central catheters [23, 24]. However, due to the lack of information on the two risk factors TPN and use of central lines were not included in the present study [1, 5, 13, 16, 25, 26].

The increase in resistance to azoles by NAC is well demonstrated in this study with *C. parapsilosis* showing resistance to both fluconazole and voriconazole, (41.2%) of isolates. Sarvikivi et al in their study demonstrated an increase in resistance to fluconazole by *C. parapsilosis* isolates [27]. *C. glabrata* that is susceptible to fluconazole requires high doses to treat it. Tarlamin et al and Ota et al both describe dose dependent susceptibility to fluconazole by *C. parapsilosis* [28, 29].The unit where the study was conducted, Amphotericin B was used to treat *C. glabrata* candidemia.

The successful use of fluconazole as prophylaxis in preventing invasive fungal infections with Candida species was reported, however a statistically significant decrease in mortality was not demonstrated[30-33]. The CMJAH neonatal unit does not give fluconazole prophylaxis to VLBW neonates. The high number of fluconazole resistant *C. parapsilosis* made the use fluconazole prophylaxis questionable.

Death as an outcome in infants with candidemia was at 28.2%, which was not statistically significantly higher than in controls. The high number of deaths in the control group is attributed to the high number of ELBW infants. Sriram demonstrated higher mortality rate in infants with candidemia 19.6% versus 10.7% for controls [34].

What the study added.

Focus was on VLBW infants who require respiratory support with NCPAP and mechanical ventilation. The study was able to demonstrate a statistically significant association between NCPAP use and candidemia. Anaemia and CLD were shown to be statistically significant which has not been demonstrated in other similar studies.

Study limitations.

The study duration was short and the statistical power was reduced due to exclusion of a significant number of infants due to missing information in the clinical records. Information on central venous catheters (CVP) and total parenteral nutrition (TPN) was missing also.
Cohort was limited to VLBW premature infants; thus the findings of the study cannot be generalised to all infants.

Conclusion.

CLD, anaemia and the need for ventilation was associated with candidemia. The increase in cases of NAC isolates was demonstrated with increasing resistance to azoles. Stricter infection control measures and medical intervention strategies to reduce the incidence of candidemia in VLBW infants should be implemented. The use of Amphotericin B in empiric treatment of candidemia should be continued in the unit and treatment only changed once results on the isolates and susceptibility patterns are obtained. A study over a longer period and a larger cohort is needed.

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Introduction.

Neonatal sepsis is an increasing cause of concern. The commonest cause of fungal blood stream infection (BSI) in the neonatal unit is Candida species. Fungal BSI is a significant cause of morbidity and mortality in neonates [1]. The mortality rate in sick neonates with fungal BSI ranges between 21% [2] and 76% [3]. In a study by Ballot et al which was conducted between January 2007 and December 2011 at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) a mortality rate of 45.8% was reported [4]. Fungal BSI is responsible for 9-13% of BIS in neonates [4]. A high index of suspicion needs to be maintained in ill neonates who remain with negative blood cultures [4]. The burden of disease and the cost of medical treatment are significant in neonates with fungal BSI and that requires implementation of strategies to minimize the burden of disease.

The group that is most likely to be affected with fungal BSI are the very low birth weight (VLBW) and extremely low birth weight (ELBW) infants, neonates born at less than 30 weeks gestational age and neonates with surgical problems [5]. The risk of neonatal sepsis is inversely proportional to birth weight and gestational age, therefore ELBW and VLBW are at increased risk of sepsis [8]. Neonates with fungal BSI present with nonspecific clinical signs. The index of suspicion should be high for neonates with sudden clinical deterioration; such as elevated or low blood glucose levels, unexplained abdominal distention, intolerance to feeds (large gastric aspirates or aspirates with abnormal discoloration), hypothermia, sudden increased requirements on ventilator settings for those on mechanical ventilation or needing supplemental oxygen whereas previously not, lethargy, platelet levels dropping and circulatory insufficiency [4, 5]. Making a diagnosis of fungal BSI can be challenging and treatment must be initiated timely. Appropriate choice of antifungal agent and timely implementation of therapy are significant with respect to patient outcomes. Currently in the unit Amphotericin B is used for presumed fungal sepsis and treatment revised based on microbiology results of organism isolated and drug sensitivity results. A delay of more than 24 hours in administration of therapy has been seen to independently increase the patient's probability of demising by two fold [9].

There are risk factors cited in the literature that predispose neonates to fungal BSI. Treatment with broad spectrum antimicrobial agents and the number of different antimicrobial agents used increases susceptibility to overgrowth of fungal microorganisms due to eradication of normal bacterial flora [4, 5].

Each time a new antimicrobial agent from a different class is used there is an exponential increase of spread of fungal BSI (4) .The VLBW and ELBW neonates invariably become exposed to more than two broad spectrum antimicrobial agents during their prolonged hospital stay and therefore may end up getting fungal BSI. Chang et al demonstrated the control of invasive Candida infections in VLBW infants by decreasing the use of 3rd and 4th generation cephalosporins in neonates with added risk factors and critical conditions [10]. These neonates are subjected to multiple venipunctures for various treatment interventions, e.g. administration of intravenous medication and fluids, total parenteral nutrition and laboratory investigations and therefore increasing the exposure to possibly colonized hands of healthcare personnel (6). The prolonged duration of stay of the inserted catheters; either peripheral or central line catheters, increases the risk of having fungal BSI (6, 7). Total parenteral nutrition(TPN) which forms a critical part of nutritional care in neonates with surgical problems is said to contribute to gut mucosal atrophy and also to be immunosuppressive thus increasing the risk of fungal sepsis (6). Some of the fungal species have a predilection for adhering to central line catheters, further increasing the risk of infection (1,4). A study by Yang et al showed that the use of TPN was a significant risk factor for fungal sepsis, the study further showed that neonates with hospital acquired blood stream infection had twice the risk of mortality (11). The lower the gestational age in the neonates (<30 weeks), the higher the risk of colonization of the skin and mucosal membranes with fungal organisms and thus increasing the risk for the invasion of the blood stream (12). Treatment with H2 receptor blockers and steroids, ventilator support and intubation with endotracheal tubes and neonates treated with nasal continuous positive airway pressure (NCPAP) were identified to be at for risk fungal BSI (4, 5). A surveillance of hospital acquired infections (HAI) by Crivaro et al conducted in Italy during 2006 - 2010 demonstrated that ELBW infants were at a higher risk of developing HAI with Candida species. *Candida albicans* and *Candida parapsilosis* were responsible for 16.3% and 10.5% respectively, out of 64% of device associated infections. The identified devices were

umbilical vein catheters, central vein catheters and mechanical ventilation [13]. Some of the mothers admitted for preterm birth do not attend antenatal care and thus miss the critical care in pregnancy, the neonates born to such mothers have been found to be at risk of fungal sepsis [5].

At CMJAH neonates with surgical problems often require central line catheters as their hospital stay is prolonged and intravenous access becomes a challenge progressively. Insertion of peripheral central lines increases the incidence of fungal BSI in the neonates who have existing central lines [7]. In the study by Ballot et al, the use and duration of central lines as a risk factor for fungal BSI were not evaluated due to lack of detailed information [4]. Prophylactic Fluconazole is not used at CMJAH following the outcome of the study by Ballot et al as the predominant fungal isolate in the study was resistant to Fluconazole, therefore making prophylaxis with Fluconazole questionable [5]. It is of paramount importance that fungal pathogen resistance and sensitivity patterns are identified and known. The selected antifungal agent must be proven to be active in vitro against the responsible pathogen [9]. Correct antifungal agent must be chosen and the administration of the antifungal agent be done within the first 24 hours of suspected sepsis [9]. Delay in therapy has been described as inappropriate treatment [9].

In CMJAH a significant number of neonates with fungal BSI is treated annually, the rate tripled in a period of 5 years from 0.6% to 1.8% between the years 2007 and 2011[5]. Shim et al looked at trends in neonatal sepsis and found fungal infections to be on the rise and they found that there is an associated increase in numbers of preterm births and increased survival of rate of VLBW babies. The study was conducted in a tertiary centre in Korea and records from 1996 to 2005 (10 years) were compared to the period from 1980 to 1995 (16 years) [14].

CMJAH neonatal unit is a large paediatric surgical referral unit, with a 14 bed shared paediatric/neonatal intensive care unit (ICU), adjacent neonatal high care with occupancy of 35 beds up to a maximum of 40 beds during busy periods, and step down neonatal care with 26 beds.

The commonest known fungal isolate in CMJAH is *Candida parapsilosis* which accounts for 54.2% of the fugal isolates, *Candida Albicans* being the next highest at

27.1% [5]. Other non- Albicans Candida (NAC) isolates are, *Candida glabrata* and *Candida tropicalis*. Amphotericin B is used as empiric antifungal cover in CMJAH, very often for prolonged periods and with undesirable side effects. Some studies report resistance to Amphotericin B, although minimal at about 3.79%, the development is concerning [5]. Kotwal et al demonstrated a shift in the pattern of fungal infections from albicans to non-albican species and a significant resistance to azoles by the fungal pathogens including C. albicans. However, newer azoles were not tested for sensitivity [15].

The type and antimicrobial sensitivity of fungal pathogens changes over time. The aim of the study is to identify the trend in neonatal fungal BSI at CMJAH, the predominant fungal isolates cultured, and to review the resistance and sensitivity patterns of the fungal pathogens. The mortality rate of neonates with fungal BSI was significant in the study conducted between January 2007 and December 2011 in CMJAH by Ballot et al. It has been four years since the study by Ballot et al therefore, it is important to have current data which can then be used to revise current health policies and antifungal treatment use. A comparison will be made between the neonates with fungal BSI and neonates without fungal BSI with relation to differences or similarities in clinical and demographic risk factor.

Study objectives.

- To describe the characteristics of neonates with fungal BSI and those without fungal BSI between 01 January 2015 and 31 December 2017 and to compare the two groups.
- To describe antifungal sensitivity and resistance patterns of isolated fungal pathogens.
- To describe the risk factors associated with fungal BSI.
- To determine outcomes of neonates with fungal BSI.

Definitions.

Fungal BSI will be defined as culture proven sepsis. The culture results and sensitivity will be obtained from the NHLS records.

Gestational age will be determined by maternal history and Ballard score.

Methods.

Inclusion criteria.

New born babies admitted to CMJAH neonate unit within 72 hours of birth.

An age of less than 28 days will define a neonate as well as less than 40 days of life for babies born prematurely and who have never been discharged home.

The study will be conducted at CMJAH neonatal department.

In the study, data from neonatal admissions between 01 January 2015 and 31 December 2017 will be reviewed. Microbiology data will be obtained from National Health Laboratory Services (NHLS) information systems (CDW).

Exclusion criteria.

Neonates with incomplete clinical records will be excluded. The number of incomplete records will be recorded.

Study design.

This is a retrospective case control study of trends of fungal BSI in neonates in the neonatal unit at CMJAH for the period of 01 January 2015 to 31 December 2017. Gender, birth weight, gestational age, duration of mechanical ventilation and NCPAP, Human Immunodeficiency Virus (HIV) exposure, antenatal care, antenatal steroids, surgical diagnosis including procedures and necrotizing enterocolitis (NEC), associated bacterial sepsis, survival to discharge, identity of fungal isolates and the predominant organism and antifungal sensitivity patterns of fungal isolates will be reviewed.

Statistics.

Data for participants will be obtained from the neonatal data base at CMJAH and NHLS microbiology laboraties for fungal isolates to correlate. The study will be powered to 80%. A two sided confidence interval of 95% will be used. Participants will be grouped according to weight categories and for each case, three controls will be chosen. Control infants will be selected as the next three babies on the same day of birth.

Data will be analysed using standard statistical methods. Continuous variables with a normal distribution will be described with mean and standard deviation, while median and range will describe skewed data. Categorical variables will be described using frequencies and percentages.

Comparison between continuous variables will be done using unpaired t-test or Mann Whitney test as appropriate (nonparametric).

Chi square analysis will be done to compare categorical variables.

STATA statistical tools will be used.

Ethics.

Permission will be sought from the hospital superintendent and authority responsible for neonatal data base in CMJAH and NHLS information systems for access to information. Patient`s identity will be concealed and separate key will be used to differentiate records.

Funding.

The study entails reviewing of records and minimal costs are anticipated and will be borne by the investigator.

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Appendix A: Author Guidelines.

Manuscripts submitted to the SAJID must be in the form of Research Articles, Brief Reports, Clinical Case Studies, Correspondence, Reviews, State-of-the-Art Articles, Commentaries and Opinion Papers, Editorials or Supplement Articles. The Journal welcomes the publication of Guidelines, Conference Proceedings Newsletters or Press Releases, and Book Reviews. Articles, Brief reports and Reviews are peer reviewed; other categories are reviewed by the Editors. Commentaries and Editorials are generally invited contributions, indicating the authors' identity, while manuscripts in the form of Reviews, and State-of-the-Art Articles may also be requested by the Editors.

All manuscripts must have conflict of interest and funding statements. When authors submit a manuscript, whether an article or a letter, they are responsible for disclosing all financial and personal relationships that might bias their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist. Authors should do so in the manuscript on a conflict-of-interest notification page that follows the title page.

Manuscripts describing research in human subjects or animals must indicate ethics clearance from appropriate research review committees. When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Articles describe original investigations at an acceptable degree of completion, constituting an advance in the field. Articles must not exceed 3500 words of text, without counting the abstract, references or legends, and illustrations and tables must be limited to the minimum necessary for clear and concise presentation. The abstract must either be structured, using Background, Methods, Results, and Conclusions as headings and comprising no more than 250 words, or unstructured

with a 200-word limit. Articles are limited to a maximum of 7 insets (tables and figures combined) and 50 references.

Brief Reports present complete studies that are narrower in scope than those described in Articles or that present new developments. Manuscripts that are descriptive or primarily methodological in nature, or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to a total of no more than 2000 words of text, a total of 2 inserts (tables or figures), and 15 references.

Correspondence (letters) must be submitted in reference to a previous publication in SAJID (within the previous 12 months), or relate to a topical matter in line with the interests of FIDSSA, PHASA or their affiliated societies. Please prepare the letter in manuscript format, including a title page. The letter must not exceed 750 words of text, 1 insert (table or figure) and 10 references.

Commentaries and Editorials are generally invited by the Editor and are overviews of articles in SAJID, or of other research in epidemiology or infectious diseases, or matters relating to public health and other issues of special interest to FIDSSA, PHASA or their associated societies. Unsolicited commentaries are also considered.

Reviews and State-of-the-Art Articles that are research oriented or fall within the fields of interests of FIDSSA, PHASA or any of their affiliated societies will be considered for publication by SAJID. Prospective authors of such manuscripts are advised to communicate with the Editor in advance to ensure that a specific contribution is deemed appropriate and timely. Manuscripts of Reviews and State-of-the-Art Articles will be peer-reviewed.

Reviewers.

The Journal would encourage authors to supply the names of at least 2 potential reviewers for their manuscript, as well as to indicate any reviewers they would feel may have a potential conflict of interest with regard to their submission.

Supplements.

Requirements for supplement manuscripts generally follow those for SAJID manuscripts, including conflict of interest and funding statements. Inquiries relating to suitability of topic, programme organisation, production and costs should be made to the Editor.

Evaluation of manuscripts.

Review procedure. The Editor-in-Chief and Emeritus Editor screen all unsolicited manuscript submissions and some of these are rejected without further review. All other manuscripts are sent to a minimum of two outside experts for

review. After receipt of the reviewers' reports, the Editor-in-Chief and the Emeritus Editor with administrative assistance of the Journal Secretary discuss the merits of the manuscripts and the Editor-in-Chief makes the final decision to accept, reject, or request revision of the manuscript. A request for revision does not guarantee ultimate acceptance of the revised manuscript

Related manuscripts. If there appears to be significant overlap between a manuscript submitted to SAJID and another submitted manuscript by the same authors to SAJID or another journal, the editors will take the matter up with the corresponding author, and based on the response, take appropriate action (ask for modification, or reject with detailed explanation). Further action may include informing the appropriate authority in the authors' resident institution and if overlapping is discovered after publication in SAJID, publishing an appropriate announcement to that effect in the journal.

DOCUMENT REQUIREMENTS.

Checklist.

The following are required for your manuscript to be processed:

 Covering letter

 Word count limits

 Conflict of interest statement

 Funding statement

 List of potential reviewers

Covering Letter.

All manuscripts submitted to SAJID must be accompanied by a letter declaring that the manuscript has not been submitted or accepted for publication elsewhere. This letter must confirm and declare that all authors have seen and approved the content and have contributed significantly to the work. Authors should suggest potential unbiased reviewers who are qualified to review their manuscript. A covering letter must also accompany a revised submission and must address issues raised in the review process.

Manuscript Preparation.

The SAJID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (Ann Intern Med 2000; 133:229-231 [editorial]; http://www.icmje.org, full text). Text, tables, references, and legends must be double-spaced. Italics should be used for genus and species names and for genes but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions. For layout of manuscript and appropriate style see a recent issue of SAJID.

Title page. On the title page, please supply a running head of not more than 40 characters and spaces, a title of not more than 160 characters and spaces, the names and affiliations of all the authors, and word counts of the abstract and text. Each author's first name, subsequent initials and surname must be used.

Footnote page. Footnotes must include:

 Statement that authors either have or have not a commercial or other association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding)

 Statement naming sources of financial support (including grant numbers)

 Name, date (month and year), and location (city, and country if not South Africa) of a meeting at which all or part of the information has been presented (include an abstract number, if available)

 Name, address, telephone and fax numbers, and e-mail address of the person to whom correspondence should be addressed

 Current affiliations and addresses for authors whose affiliations have changed since completion of the study

Abstract. The abstract for an Article may be structured with the headings Background, Methods, Results, and Conclusions (250-word limit) or unstructured (200-word limit). Abstracts of Brief Reports should be no more than 100 words. Whether structured or unstructured, the abstract must state the purpose of the research, the methods used, the results, and the conclusions. Do not cite references in the abstract. Include up to 10 key words, separate from the abstract. Please remember that the abstract is particularly useful for literature retrieval purposes.

Text. The text of Articles must be no longer than 3500 words, and that of Brief Reports no longer than 2000 words. The Methods section must include a statement that informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the National Department of Health (http://www.doh.gov.za) or the South African Medical Research Council (MRC; http://www.sahealthinfo.org/ethics/index.htm) and /or those of the authors' institution(s) were followed in the conduct of clinical research or that animal experimentation guidelines (see MRC website above) were followed in animal studies.

References. Articles are generally limited to 50 references, Brief Reports to 15 references. Only works that have been published or accepted for publication can be included in the reference list. Unpublished observations by the authors (authors' unpublished data) personal communications (SP Stanley, personal communication), and manuscripts submitted for publication (J Odendaal, S Coovadia and J Radebe, submitted) should be mentioned parenthetically in the text Please number references in order of appearance; those cited only or first in tables or figures are numbered

according to the order in which the table or figure is cited in the text. Example: If table 3 is cited in the text after reference 20, a new reference cited in table 3 will be reference 21.

References must follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org, full text). Provide all authors' (or editors') names when there are fewer than 7; for 7 or more, list the first 3 and add "et al." Titles of journals not listed in Index Medicus should be spelt out in full. Reference to a doctoral thesis or Master's dissertation should include the author, title, institution, location, year and publication information, if published. For online resources, include a URL and date accessed. Accuracy of references is the responsibility of the authors.

Examples of the proper format are as follows:

Sonnenberg P, Glyn Thomas R, Glynn JR, Shearer S, Godfrey-Faussett, Murray J. Clinical and radiological features of pulmonary disease due to culture-positive Mycobacterium tuberculosis or non-tuberculous mycobacteria in South African gold miners. South Afr J Epidemiol Infect 2005; 20: 130-135

Marin M, Nguyen HQ, Langidrik JR, et al. Measles transmission and vaccine effectiveness during a large outbreak on a densely populated island: Implications for vaccination policy. Clin Infect Dis 2006; 42: 315-319

Strebel PM, Papania MJ, Halsey NA. Measles vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. 4th ed. Philadelphia: WB Saunders, 2004: 389-440.

Mothibeli KM, McGee L, Smith AM, Klugman KP. Molecular epidemiology of pneumococcal serotype 3 isolates. [abstract ID P56]. In: Programme and Abstract Book of the 1st Joint Congress of the Federation of Infectious Diseases Societies of Southern Africa (Sun City, North-West Province). Johannesburg: Presentations Graphics, 2005: 42.

World Health Organization. Initiative for vaccine research. Available at: http://www.who.int/vaccine_research/diseases/measles/en/. Accessed 1 February 2005.

Acknowledgment(s). The page preceding the references may include a statement thanking those who assisted substantially with work relevant to the study.

Statistical analysis. The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of measure. All Data should be expressed in metric units; use of SI units is encouraged. Use ºC for temperature.

Tables and figures. Articles are limited to a maximum of seven inserts (tables and figures combined), Brief Reports to a maximum of two inserts. Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, even if already defined in the text.

Tables should be numbered in the order of mention in the text. Tables should be typed double-spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory material should be kept to a minimum. Footnotes should be placed below the table and designated by superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word, consult the Special Instructions for Tables.

Figures should be also numbered in the order of mention in the text and should appear at the end of the manuscript and references. Your figures should be prepared in accordance with the Guidelines for Submission of Artwork. Letters, numbers, and symbols should be clear and of sufficient size to be legible when the figures are reduced. Photomicrographs should have internal scale markers. Figures reproduced from other publications must be accompanied by permission from the copyright holder. If the manuscript is accepted, the author will be required to send one complete set of glossy, hard-copy figures.

Figure legends should be double-spaced and appear on a separate page preceding the figures. Any abbreviations or symbols used but not defined in the figure itself must be defined in the legend.

Style. Authors are referred to the American Medical Association Manual of style: A Guide for Authors and Editors (9th ed., Williams& Wilkins, 1997) and the Chicago Manual of Style (15th ed., University of Chicago Press, 2003).

For commercially obtained products mentioned in the text, list the full names of manufacturers. Generic names of drugs and other chemical compounds should be used.

Nomenclature. SAJID recommends the latest widely accepted nomenclature, as set out in documents prepared by recognised international agencies e.g. the International Journal of Systematic and Evolutionary Microbiology, Bergey's Manual of Determinative Bacteriology (9th ed., revised, Williams& Wilkins, 1993), Virus Taxonomy – The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses (Springer-Verlag,1995). The latter document also supplies standard abbreviations for virus species.

Clinical trials registration. All clinical trials must be registered in a registry that is electronically accessible to the public, free of charge. Registration should occur before patient enrolment and the registry's URL and the trial's registration number must be supplied at the end of the manuscript's abstract. For information on acceptable registries, consult the ICMJE Web site, http://www.icmje.org. The National Library of Medicine's registry which is free and open to all investigators, generally meets with the requirements of journals for the publication of clinical trials.

MANUSCRIPT SUBMISSION.

Procedure.

Authors are advised to retain a copy of submitted manuscripts, including tables, figures and photomicrographs. The journal is not responsible for manuscripts lost or damaged.

All manuscripts must be submitted online at www.sajei.co.za. Register as an author, login in and click on CLICK HERE TO FOLLOW THE FIVE STEP SUBMISSION PROCESS. The covering letter must please be submitted as a supplementary file.

For assistance to upload your manuscript or further instructions please contact Ms Robyn Marais at toc@sajei.co.za.

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R14/49 Dr Carol Malunga and Dr Trusha Nana

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CLEARANCE CERTIFICATE NO. M151190

NAME: (Principal Investigator)	Dr Carol Malunga and Dr Trusha Nana
DEPARTMENT:	Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Trends in Neonatal Blood Stream Fungal Infections at Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	27/11/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Daynia Ballot
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/12/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

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

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**

Principal Investigator Signature — Date 02/06/16

02/06/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPEDIX D: Plagiarism declaration.

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS SENATE PLAGIARISM POLICY:

I Carol Jacobeth Malunga, Student number: 1261378 am a registered student for the degree of Masters of Medicine, Paediatrics in the 3rd year of academic studies. I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

I have included as an appendix a report from "TURNITIN" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: _____