A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the neonatal ICU and high care unit in Universitas Academic Hospital over a 2 year period from January 2016 to December 2017 to determine if the unit will qualify for the use of fluconazole prophylaxis.

By

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"I, Leandri de Klerk, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Paediatrics 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."

Signed at Bloemfontein on the 1st of June 2020

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Abstract

Background: Invasive Candida infection is a leading cause of mortality and morbidity during the neonatal period, specifically in premature neonates admitted to intensive care units. Fluconazole prophylaxis has been proven to effectively reduce the incidence of invasive candidiasis in neonates admitted to high care facilities.

Methods: A retrospective, descriptive, cross-sectional, non-experimental study was conducted. Data was collected from the Meditech and National Health Laboratory Service databases. Positive culture results on sterile body fluids were analyzed to determine the incidence of invasive candidemia in the neonatal unit at Universitas Academic Hospital. Sterile body fluids included blood, urine and cerebrospinal fluid.

Results: The results revealed an incidence of 13.5% of invasive candidiasis in the extremely- and very low birth weight neonates admitted to the neonatal unit at Universitas Academic Hospital. These results included all the positive cultures done on urine, cerebrospinal fluid and blood. The study population was 324 neonates admitted to the neonatal unit of Universitas Academic Hospital with a birth weight of less than 1.5 kg for the years 2016 and 2017. The population group included 183 female neonates (56%) and 141 male neonates (44%). The median gestational age was 29 weeks and the median birth weight was 1110g. The positive Candida culture results included 43 blood cultures, 5 urine cultures and zero cerebrospinal fluid cultures.

Conclusion: The incidence rate for invasive candidiasis in very low birth weight and extremely low birth weight neonates at Universitas Academic Hospital was 13.5% during the study period. Previous research studies concluded that an incidence rate higher than ten percent of invasive Candida infection is significant and a neonatal unit will benefit from the use of fluconazole prophylaxis. Decreasing the incidence will have a direct effect on the morbidity associated with neonatal invasive Candida infection. The recommendation following the findings of the study would be to include the use of fluconazole prophylaxis as standard

of care in the neonatal unit at Universitas Academic Hospital and to adapt policies and protocols accordingly.

Keywords and definitions

Neonate: Classified as a baby from birth to 28 days of life.

Fluconazole: Anti-fungal medication used to treat fungal infections.

Prematurity: Infants born before 37 completed weeks of gestation.

Prophylaxis: Treatment given in order to prevent a certain disease or illness.

Incidence: Total number of new cases of a specific disease or illness reported in a specific unit in a specific period.

Extremely low birth weight: Neonates born with a birth weight of less than 1kg.

Very low birth weight: Neonates born with a birth weight between 1kg and 1.5kg.

Invasive candidiasis: A positive culture for Candida on any sterile fluid of the body, mainly urine, cerebrospinal fluid (fluid surrounding the brain and spinal cord) and blood.

Meditech: Database used by Universitas Academic Hospital to store patient information and note keeping.

List of abbreviations

CLD:	Chronic lung disease
CMSA:	Colleges of Medicine of South Africa
CSF:	Cerebrospinal fluid
HPCSA:	Health Professions Council of South Africa
HSREC:	Health Sciences Research Ethics Committee
ICS:	Invasive candidiasis
NHCU:	Neonatal High Care Unit
NHLS:	National Health Laboratory Service
NICU:	Neonatal Intensive Care Unit
PVL:	Periventricular leukomalacia
ROP:	Retinopathy of prematurity
UAH:	Universitas Academic Hospital
VLBW:	Very low birth weight
ELBW:	Extremely low birth weight

Chapter 1

Neonatal sepsis remains one of the leading causes of mortality in the neonatal unit. Bacterial sepsis followed by invasive candidiasis are the two leading causes of neonatal sepsis. (1) According to South African data neonatal sepsis is under the top three causes of neonatal mortalities. (62) The premature neonate has the highest risk of developing sepsis. Other risk factors associated with an increased risk to develop neonatal sepsis and specifically invasive candidiasis include the use of total parenteral nutrition, mechanical ventilation, use of broad spectrum antibiotics and proton pump inhibitors, gastrointestinal surgery and the insertion of central venous lines. (2,3,6) The mortality caused by invasive Candida infection in neonatal units ranges between 25-50%. (1,8,23)

Premature neonates, specifically those with a very low birth weight (VLBW) and extremely low birth weight (ELBW), are largely at risk for the development of invasive candidiasis (ICS), due to their immature immune system. (4,5) The immune system is built out of the chemical and physical barriers in the epithelial and mucous membranes, next in line to protect against unwanted organisms is the non-specific (innate) and the specific (adaptive) components of immunity. Innate immunity is the first line of defense and can be divided into the humoral and cellular response. The humoral response includes complement, interferon, lactoferrin and lysozymes that destroy the unwanted organism. The cellular response that consists primarily of phagocytic white cells such as neutrophils, macrophages, monocytes and natural killer cells are released in a response to the inflammatory cascade produced by the tissues when infected or damaged. The phagocytic cells adhere and ingest the bacteria and then enzymes and free oxygen radicals are released to destroy the bacteria. Adaptive immunity is mediated through antibodies produced by the B-lymphocytes and the specific cytotoxic cells produced by the T-lymphocytes. Antibodies consist out of immunoglobulins with a main function to protect against infections. Cytotoxic cells are responsible for killing infected cells and preventing viral replication. (61)

The immune system develops from early fetal life, but is only fully functional by

one year of age. There are multiple factors that increase the risk for infection in the neonate. Premature neonates have underdeveloped layers of skin. Exogenous factors such as breaches of the skin barrier by cannulas or venipuncture may allow entry of bacteria and they are predisposed to be contaminated with potentially pathogenic organisms. Endogenous factors include decreased numbers and function of neutrophils, complement, phagocytic cells, immunoglobulins and lymphocytes. Premature neonates fail to receive normal passive immunoglobulin transfer during the last trimester of pregnancy. (61)

They have a high risk to develop necrotizing enterocolitis and intestinal perforation, requiring central venous lines, broad spectrum antibiotics and total parenteral nutrition. Each of these factors affects the physiological barriers, which increases the risk of developing invasive candidiasis. (30)

The most common complications associated with ICS include shock and renal failure. Other targeted sites of infections and complications are the central nervous system which include meningitis and cerebral abscesses, soft and deep tissue abscesses, heart (endocarditis), eyes (endophthalmitis), lungs (pneumonitis) and liver (hepatitis). (24,31) After surviving ICS, the neonates are at higher risk of developing retinopathy of prematurity (ROP), periventricular leukomalacia (PVL) and chronic lung disease (CLD). (7) In comparison with neonates whom did not have ICS, the neonates whom did have ICS showed a global delay in neurological development at the age of 18 months. (21)

Candida albicans is the most prevalent fungal species to cause disease in the neonate. Other common species that cause neonatal disease are Candida parapsilosis, Candida glabrata, Candida tropicalis, Candida krusei and Candida lusitaniae. (30,32-35)

The incidence of invasive candidemia escalated because of the increased survival of the very low birth weight and the extremely low birth weight neonate in current units. (25) The advances in management of surgical conditions in the neonate also contribute to development of fungal infections. (26) The highest incidence of invasive disease in the neonate has been recorded between the second and sixth

week after birth. (24,31)

The mouth, skin and gastrointestinal system of neonates are colonized with Candida that increases the risk to develop invasive candidiasis. (27) Seven to twenty percent of premature neonates that are colonized with Candida, develop invasive disease. (4,27,28) Other modifiable factors that increase the risk are indwelling catheters or tubes, overuse of third generation cephalosporin antibiotics, hospital staff that spread the infection due to poor hand hygiene and overcrowding in a unit.

The colonization may be secondary due to vertical transmission from the mother or horizontal transmission due to nosocomial infection. Studies have shown that 4.8-10% of neonates are colonized with some strain of Candida. (36) If admitted to a NICU, 50% of neonates can become colonized with Candida within the first week of stay and 64% by four weeks. (37,38) The vertical transmission route is the most common route of transmission for Candida albicans, where Candida parapsilosis accounts for the most nosocomial infections. (38,39) In one multicenter study Candida were isolated from 29% of health workers (859 of 2989). Candida albicans (19%) and Candida parapsilosis (5%) were the fungal species predominantly isolated. (3,39)

Invasive Candida infection usually cause late onset neonatal sepsis and carries a high mortality and morbidity risk. (8,1) After being diagnosed with invasive candidiasis, the treatment with intravenous anti-fungal therapy is usually a minimum of 14–21 days. (16) This leads to a prolonged hospital stay and increased cost to the patient and/or hospital.

Blood cultures remain the gold standard of diagnosing infections in neonates, but do have a very low sensitivity for invasive candidiasis in adults (28%) and even lower in neonates. Thrombocytopenia occurs in 80% of neonates with invasive candidiasis, but is not specific only to Candida sepsis. Thus making it very challenging to diagnose fungal infections. A decision to initiate empirical anti-fungal medication should be based on a high index of suspicion, non-specific clinical signs and symptoms and risk factors for each patient individually. (30)

New methods are being studied to assist in early diagnosis of invasive Candida infection, because of the blood culture results that can take up to three days before testing positive, as well as the low yield to culture these organisms and non-specific signs and symptoms that neonates present with when having a Candida infection. One of these tests is the (1,3)-beta-D-glucan assay to help detect and diagnose Candida infections. (40) Beta-D-glucan (BDG) is a component of the fungal cell wall and performed well to identify invasive disease in the adult population, although the performance in the neonatal population are not yet well understood. (41-45) A meta-analysis revealed sensitivity of 57-97% and specificity of 56 - 93% when using the (1,3)-beta-D-glucan assay to assist with the diagnosis of invasive candidiasis. (46) A retrospective study done on forty-seven neonates in 2018 found that the (1,3)-beta-D-glucan assay did diagnose invasive fungal infections (sensitivity of 61.5% and specificity of 81%) and that the trend could also be used to evaluate treatment progress. (40) Another retrospective study done on eighty neonates in China did show an association between high level of BDG and invasive Candida infection. (47) Further research on larger population groups are needed to produce evidence that is more significant.

Treatments of invasive disease include systemic usage of an antifungal drug, prompt removal and replacement of central venous catheters, control of predisposing underlying condition and assessment of deep tissue infection and complications. (30,48) Delayed removal of central catheters was associated with an increase in mortality rates. Anti-fungal therapy should be continued fourteen to twenty-one days after the first negative culture result. Amphotericin B deoxycholate has been the mainstay of treatment for invasive Candida infection. Side effects include renal toxicity and electrolyte abnormalities. The lipid formulation of amphotericin B is associated with worse outcomes in neonates and should be used with caution. (30) Fluconazole is an azole with a long half-life, good cerebrospinal fluid penetration and good levels in the tissues, blood and body fluids. Currently fluconazole is considered as a good alternative drug to use for invasive fungal infection if amphotericin B is not available or if contra-indicated. (49,50)

Research suggest that if the incidence of fungal sepsis in a neonatal unit is higher than 10%, that the use of fluconazole prophylaxis at a dosage of 3-6mg/kg twice weekly for a total period of three weeks can decrease the incidence of invasive candidiasis in that unit. (9-13,15) Initially research recommended fluconazole at a dosage of 3-6mg/kg as a daily dosage starting in the first three days of life and to continue until three to four weeks of life. Thereafter research concluded that the usage of fluconazole at a dose of 3-6mg/kg twice weekly showed the same efficiency and reduction in fungal infections in the high-risk group neonates. (51,52) The incidence of invasive Candida infection ranges between 2-4% and can go up to 16% in extremely low birth weight neonates. (9,15) There is little evidence that shows that fluconazole prophylaxis has an effect on the reduction in the mortality rate caused by invasive Candida infection, but the morbidity caused by invasive candidiasis is decreased. (9,15)

No immediate side effects were reported or uncovered in the studies where fluconazole prophylaxis was given to the patients; the drug was reported as safe for usage as prophylaxis in the neonate. (22) Concerns were raised about the possibility to develop fluconazole resistance, especially the innate fluconazole resistant species like Candida glabrata and Candida krusei. No increase in number of resistant organisms was observed in five major neonatal prophylaxis studies. Furthermore, there was no increase reported in the incidence of innate fluconazole resistant fungus as mentioned previously. (53,54) Fluconazole prophylaxis had no effect on liver function tests. When used for more than four weeks it may have an influence on the resistance profile of the Candida species. (19) Follow up of the patients who received four weeks of treatment with fluconazole prophylaxis did not show any negative impact on the neurological development of the patients. (20)

Other alternatives described in literature to prevent fungal sepsis are daily oral nystatin or probiotics. Previous small studies investigated oral nystatin as prophylaxis for fungal infections and a reduction in fungal bloodstream infection was shown. Another recently documented randomized, controlled trial compared oral fluconazole prophylaxis with nystatin prophylaxis. Invasive disease was

diagnosed in 5.3% of the fluconazole group versus 14.3% in the nystatin group. Mortalities caused by perforation and necrotizing enterocolitis were associated with the use of nystatin oral treatment; good safety data are lacking for the use of nystatin in neonates as prophylaxis and its use is not recommended according to literature. (55-57)

With the usage of broad spectrum antibiotics, the bacteria lining the gut usually are eliminated, this disrupts the mucosal barrier and increases the risk for infection. Antibiotics eliminate bacteria, but leave fungi unaffected. This offers Candida an opportunity to proliferate and cause opportunistic infections. Early restoration in the microbial flora of the gut can prevent infection. Oral use of probiotics modifies the enteric flora by colonizing the gastrointestinal system and preventing overgrowth of unfavorable organisms. (58,59) Probiotics have been studied and shown to be beneficial in preventing invasive fungal sepsis and colonization in mice models. According to a systematical review done in 2016 it was found that probiotics did reduce Candida colonization in the preterm neonate in a NICU, but did not decrease invasive disease. Probiotics however have been associated with systemic infections and are not routinely prescribed due to lack of safety data. (60) In conclusion fluconazole is superior to the other two options to be used for prophylaxis as supported by robust clinical data.

Decreasing the incidence of invasive candidiasis in a unit, the unit will be less colonized with Candida and secondary to that the overall sepsis risk will also decrease. (14) There is an association between ICS and bacterial sepsis. It has been documented that a decrease in ICS also reduces the bacterial sepsis rate. (17,18) By decreasing the overall sepsis in the unit, the length of stay and usage of antibiotics will be decreased. This has clear financial implications for the healthcare system.

Nevertheless, the use of fluconazole prophylaxis is not standard care in all the neonatal units in different countries. In an attempt to possibly implement this at Universitas Academic Hospital, the incidence of invasive candidemia in the neonatal HCU and ICU at this hospital was calculated to evaluate if this unit will qualify and benefit from the use of fluconazole prophylaxis.

The purpose of the study was to determine the incidence of invasive candidiasis in the neonatal unit of Universitas Academic Hospital. The study focused on the positive cultures on blood, urine and cerebrospinal fluid for Candida species during the years 2016 and 2017 in the extremely low birth weight and the very low birth weight neonates admitted to this specific unit. The secondary objective was to determine whether this unit would qualify and benefit from the use of fluconazole prophylaxis.

The high sepsis rate at the neonatal unit at UAH is concerning and contributes significantly to morbidity and mortality. Challenges such as staff shortages, overcrowding and lack of consumables increase the incidence of sepsis in this unit. According to the monthly statistics that are presented at UAH, rates of sepsis are high (no specific number calculated) and several organisms have been cultured in the unit. To date, no specific incidence rates have been calculated to determine the contribution of invasive candidiasis to the unit.

There were a few limitations that affected the study. The first is that eighty babies died during the years of 2016 and 2017 that were also included in the population group. Most of these patients were ELBW, making them high risk patients to have developed invasive candidiasis during the course of admission in the neonatal unit. This could have affected the results and the incidence rate could maybe have been calculated at an even higher rate. This study did not focus on the cause of death; subsequently invasive candidemia could not be linked as a cause of these mortalities.

Another limitation could be the quality of specimen cultures influenced by the technique of the health care worker, sterility of the procedure and volume of sterile body fluid inserted into the culture bottles or specimen tubes. The sensitivity of blood cultures for Candida in adults is only 50%, when the adult already has multi-organ involvement secondary to the fungal infection. (16,29) This is even lower in neonates, due to smaller volumes of blood inserted in the blood culture bottle. (16,29)

The third limitation was that the cultures were not always drawn on the day that the infection was suspected, occasionally the health care workers delay or defer taking specimens for cultures and there after lack to perform follow up cultures. Thus patients may already have been on empiric anti-fungal medication influencing the yield of positive cultures.

This results opens up doors for many studies that can be embarked upon in the future. The follow up incidence rate will need to be calculated after the start of fluconazole prophylaxis to determine if the unit did benefit from the use of the prophylaxis. Also the neonates need to be followed up at 18 months of age to monitor neurodevelopmental outcome. Additional information can be investigated further. This includes the resistance profile of Candida species after initiating fluconazole prophylaxis, the effects of fluconazole as prophylaxis used over a shorter period as three to four weeks and possible side effects that were identified in the neonates while using fluconazole as prophylactic treatment. It would also be of interest to determine the incidence of ICS in the neonatal unit at Pelonomi Tertiary Hospital.

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Chapter 2

Abstract

Background: Invasive Candida infection is a leading cause of mortality and morbidity during the neonatal period, specifically in premature neonates admitted to intensive care units. Fluconazole prophylaxis has been proven to effectively reduce the incidence of invasive candidiasis in neonates admitted to high care facilities.

Methods: A retrospective, descriptive, cross-sectional, non-experimental study was conducted. Data was collected from the Meditech and National Health Laboratory Service databases. Positive culture results on sterile body fluids were analyzed to determine the incidence of invasive candidemia in the neonatal unit at Universitas Academic Hospital. Sterile body fluids included blood, urine and cerebrospinal fluid.

Results: The results revealed an incidence of 13.5% of invasive candidiasis in the extremely- and very low birth weight neonates admitted to the neonatal unit at Universitas Academic Hospital. These results included all the positive cultures done on urine, cerebrospinal fluid and blood. The study population was 324 neonates admitted to the neonatal unit of Universitas Academic Hospital with a birth weight of less than 1.5kg for the years 2016 and 2017. The population group included 183 female neonates (56%) and 141 male neonates (44%). The median gestational age was 29 weeks and the median birth weight was 1110g. The positive Candida culture results included 43 blood cultures, 5 urine cultures and zero cerebrospinal fluid cultures.

Conclusion: The incidence rate for invasive candidiasis in very low birth weight and extremely low birth weight neonates at Universitas Academic Hospital was 13.5% during the study period. Previous research studies concluded that an incidence rate higher than ten percent of invasive Candida infection is significant and a neonatal unit will benefit from the use of fluconazole prophylaxis.

Decreasing the incidence will have a direct effect on the morbidity associated with neonatal invasive Candida infection. The recommendation following the findings of the study would be to include the use of fluconazole prophylaxis as standard of care in the neonatal unit at Universitas Academic Hospital and to adapt policies and protocols accordingly.

Introduction

Neonatal candidemia is the second most common infection found in neonatal units. Bacterial infections are the leading cause of infections in neonates. Invasive candidemia carries a high burden of mortality ranging between 25-50%. (23) Neonates that do survive after being treated with anti-fungal medication tend to have neurodevelopmental delay at the 18 month neurological assessment. (21)

After being diagnosed with invasive candidiasis, a neonate requires prolonged intravenous treatment for 14-21days with an anti-fungal drug. (16) This increases the financial burden on the hospital. Patients also need to be screened for several end-organ complications as invasive candidiasis could involve multiple organ systems including the brain, kidneys and heart. (24)

The incidence of invasive candidemia has escalated because of the increased survival of the very low birth weight and extremely low birth weight neonate due to improved perinatal and neonatal practices. (25) The advances in management of surgical conditions in the neonate also contribute to the increased rate of development of fungal infections. (26)

The mouth, skin and gastrointestinal system of neonates are colonized with Candida that in itself increases the chance to develop invasive candidiasis. (27) Seven to twenty percent of all preterm neonates who are colonized with Candida, develop invasive disease. (4,27,31) Other modifiable factors that increase the risk are indwelling catheters or tubes, overuse of third generation cephalosporin antibiotics, hospital staff that spread the infection due to poor hand hygiene and overcrowding in a unit.

According to multiple randomized controlled trials and retrospective cohort studies the use of fluconazole prophylaxis in neonatal ICU's with a high incidence of invasive candidemia has shown to reduce the incidence. (4,28,29,30)

The safety profile of fluconazole has also been studied in depth. In one study, patients who were followed up at 18 months and also at 8 years of age had no long term side effects and no negative effect on neurodevelopment was found. (19,20,22,32)

Nevertheless, the use of fluconazole prophylaxis is not standard care in all the neonatal units in different countries. In an attempt to implement this at Universitas Academic Hospital, the incidence of invasive candidemia in the neonatal HCU and ICU at this hospital was calculated to see if this unit will qualify and benefit from the use of fluconazole prophylaxis.

Methods

Study design

This was a retrospective descriptive cross sectional study. This study design enabled the researcher to collect the positive Candida culture results and calculate the incidence in this unit.

Target population and population size

All neonates with a birth weight below 1.5kg and admitted to Universitas neonatal ICU and HCU from 1st January 2016 to 31st December 2017 were included in the study. A total of 324 neonates were included.

Data sources

Data was collected from the following databases: Meditech, NHLS and Department of Microbiology records. NHLS and Microbiology provided the positive cultures for Candida species in the neonatal unit for the years 2016 and 2017. After collecting all the positive culture results, the profile of the patients was determined using the hospital number on Meditech to identify the weight category, gender and age at onset of the infection. All the neonates with a birth weight below 1.5kg were documented even if they had a negative culture, in order to calculate the incidence; both positive and negative results were captured.

A data-capturing sheet was used to record the necessary information needed for the research study and also the culture results. The data was analyzed with the help of a biostatistician.

Information was gathered to definitely answer the research question. In order to prevent bias, unnecessary data was not collected. This is in keeping with other studies with the same aim, specifically to determine the incidence of invasive candidemia.

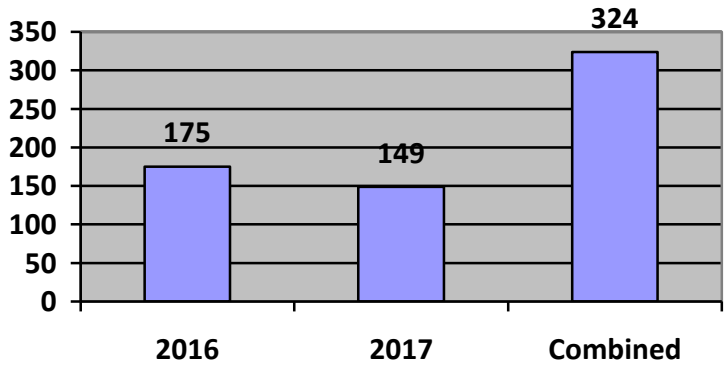
The confidentiality of patient information was prioritized. Only the researcher handled the patient's files and data capture sheets in a private and secure room. A study number was allocated to each patient to protect his or her identity.

Results

Population characteristics

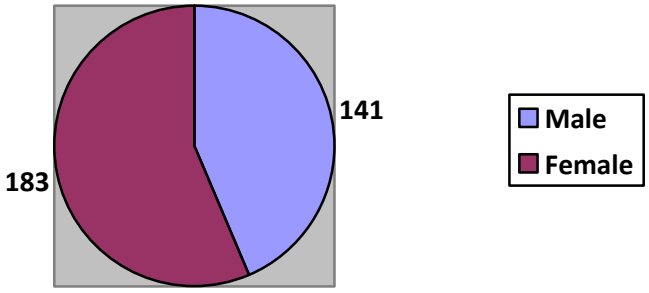
Included in the study was a population size of three hundred and twenty-four neonates who were admitted to the neonatal unit of UAH with a birth weight below 1.5 kg for the years 2016 and 2017.

Graph A:
The total of neonates included in this research project



The total study population included 183 (56%) female neonates and 141 (44%) male neonates. There was no statistically significant difference in the gender distribution of the study population.

Graph B:
Gender Distribution



The median gestational age was twenty-nine weeks and the median birth weight was 1110g.

Culture results

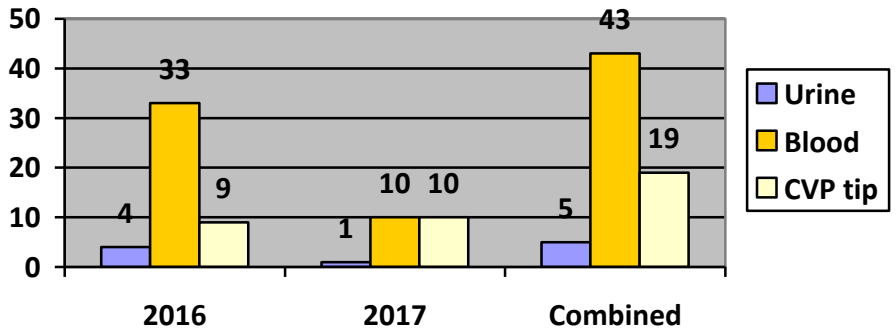
A total of forty-three blood cultures tested positive for Candida species.

Five urine cultures tested positive for Candida.

Zero CSF cultures tested positive for Candida.

An interesting incidental finding during collecting data, was that nineteen central venous catheter tips tested positive for Candida in this unit.

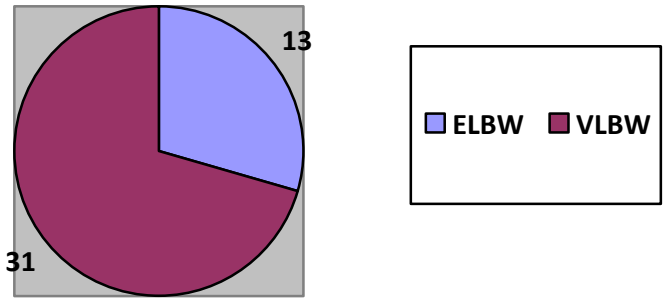
Graph C:
Candida Positive Culture Results



Four of the five urine cultures were from patients who also had a positive blood culture for Candida. These were only counted as one positive culture per patient. The total number of positive Candida cultures on blood, urine and CSF were forty-four. Calculating the incidence of invasive Candidiasis for 2016 and 2017 at 13.5%.

Out of the 44 positive results, 13 (29.5%) had a birth weight below 1kg and 31 (70.5%) had a birth weight between 1kg and 1.5kg.

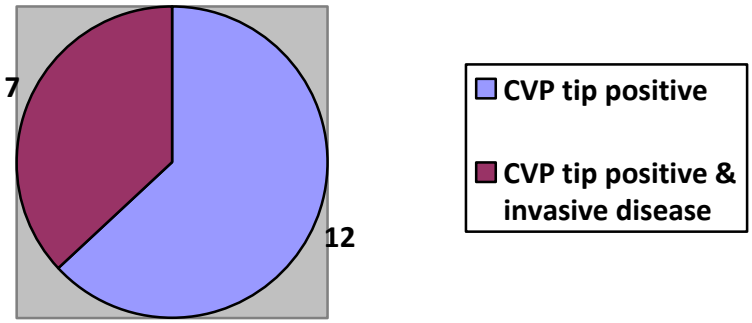
Graph D:
Birth Weight Categories



The median age at onset of invasive candidiasis in the neonates was on day nineteen of life. The youngest neonate being seven days of life and the oldest neonate was forty-seven days old at onset of invasive disease.

An interesting incidental finding during collecting data, was that nineteen catheter tips tested positive for Candida in this unit. Only seven out of the nineteen patients that had a positive culture on their CVP tip had invasive disease, indicated by a positive blood culture as well.

Graph E:
Positive CVP tip cultures



Of the forty-four patients with invasive Candida infection, thirteen also had multiple other bacterial infections proven by blood cultures taken on different occasions.

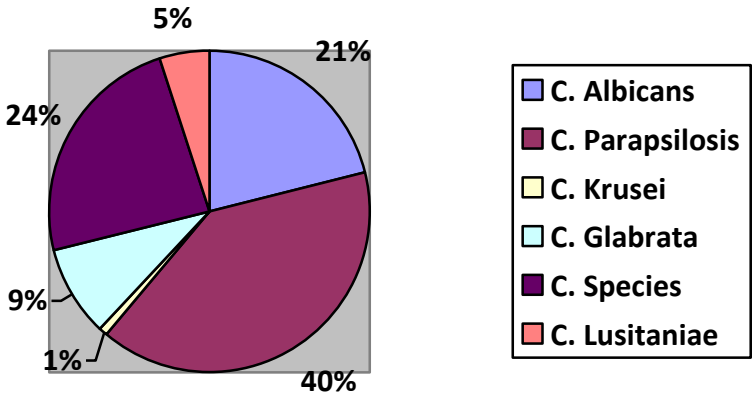
Table A:

Comparison between Candida species cultured

	Blood	CVP tips	Urine	Total
Candida albicans	10	3	1	14
Candida parapsilosis	19	8	0	27
Candida krusei	1	0	0	1
Candida glabrata	4	2	0	6
Candida species	6	6	4	16
Candida lusitaniae	3	0	0	3

Graph F:

Variety of Candida species



Discussion

The incidence of invasive candidiasis in the VLBW and ELBW neonates in the neonatal unit of UAH is 13.5%. In comparison with previous studies done in other countries, an incidence rate of higher than 10% is concerning and these units qualify to use fluconazole as prophylaxis to decrease the incidence. (15) As mentioned earlier in the literature review, Candida infection carries a high mortality and morbidity rate and should be prevented as far as possible.

The median age at onset of invasive Candida infection was nineteen days of life. The risk of invasive candidiasis extends from birth until three months of age, but the highest risk is between eleven and twenty days of life. (1) Thus our data compares well with previous studies.

An interesting incidental finding, was that nineteen catheter tips tested positive for Candida. The incidence of catheter tips that tested positive for Candida was 5% and could indicate that the unit or the neonates are colonized with Candida or that the personnel that inserted the central lines failed to use a proper sterile technique putting these neonates at high risk to develop invasive disease. This hypothesis might be supported by the literature on colonization of neonates, NICU's and healthcare workers. (3, 35-38) However, further investigations into this finding needs to be done in order to prove the hypothesis and enable appropriate corrective measures. Only 36% of the patients that had a positive CVP tip had invasive disease. Therefore adhering to strict sterile techniques while inserting central lines should be emphasized. Other infection control measures to prevent colonization, like proper and regular hand washing, should also be adhered to. A recent meta-analysis done in September 2018 showed a statistically significant reduction in central line associated bloodstream infections (CLABSI) following the introduction of care bundles when inserting central lines in neonatal units. This equated to 60% reduction in CLABSI. (34) Care bundles are usually focused on a few points to prevent CLABSI. These factors are hand hygiene, critical indication for central lines and removal as soon as possible, skin asepsis and cleaning regularly with anti-septic medium during dressing changes, maximum barrier

precautions and strict sterile techniques when inserting central lines and regular cleaning as well as replacement of infusion systems, ports and connections. (34)

The majority of the study population who had invasive candidiasis had a birth weight between 1kg and 1.5kg, which contradicts the literature that states that the ELBW neonate are at the highest risk to develop invasive disease. This finding could be due to the eighty babies that were included in the study population, which died during the years of 2016 and 2017. Most of these patients were ELBW, making them high risk patients to have developed invasive candidiasis during extended admission in the neonatal unit. This could have affected the results and the incidence rate could maybe have been calculated at an even higher rate. The study did not focus on the cause of death; subsequently invasive disease could not be linked as a cause of these mortalities.

Another limitation could be the quality of specimen cultures and the way it was collected; some techniques could have influenced the results. The sensitivity of blood cultures for Candida in adults is only 50%, despite already having multi-organ involvement secondary to the fungal infection. This is even lower in neonates, due to smaller volumes of blood inserted in the blood culture bottle. (16,33) This makes the case for standard fluconazole prophylaxis even stronger.

This study did not focus on the resistance profile of the Candida species cultured in the neonatal HCU and ICU, it mainly focused on the incidence of ICS. Follow up studies can be done to determine the resistance profile and if fluconazole prophylaxis had an effect on the results. Research showed no increase in number of resistant organisms observed in five major neonatal prophylaxis studies. Furthermore, there was no increase reported in the incidence of innate fluconazole resistant fungus as mentioned previously. (19,39)

Literature has shown that if the incidence is higher than 10% in a neonatal unit, the unit will benefit from the use of prophylaxis. Fluconazole prophylaxis is given at a dosage of 3-6mg/kg twice weekly for three weeks per mouth or intravenous. There has been no side effects or poor long term neurodevelopmental outcomes linked to the usage of this medication as prophylaxis. (9-13, 15)

These results open up doors for many research opportunities to be explored in the future. The follow up incidence rate should be calculated after the start of fluconazole prophylaxis to determine if the unit did indeed benefit from the use of the prophylaxis as expected. Also the neonates who received fluconazole prophylaxis need to be followed up regularly and specifically at 18 months of age to monitor neurodevelopmental outcomes. A replication of the study at the neonatal unit at Pelonomi Tertiary Hospital is encouraged to determine whether fluconazole prophylaxis should possibly become standard practice.

This study focused on the VLBW and ELBW categories to determine the incidence of ICS, as this group of neonates have been identified to be high risk and to benefit from prophylaxis. However, Candida sepsis is not mutually exclusive to this group; therefore, future studies could look at the incidence in all neonates to determine the overall incidence in the unit. This would allow the evaluation of the overall decrease in ICS after the introduction of prophylaxis in the high risk group. It would be interesting to see if, by providing prophylaxis to the VLBW and ELBW neonates, it will affect the incidence of ICS in other neonates, suggesting a decrease in colonization of the neonatal unit.

Conclusion

The neonatal unit at UAH had a 13.5% incidence rate for invasive candidiasis in the VLBW and ELBW neonates for the year 2016 and 2017. According to literature an incidence rate of more than ten percent in the unit will benefit from the use of fluconazole prophylaxis. Therefore, this unit would qualify for the use of prophylaxis and it is recommended that policies and protocols be adapted to incorporate fluconazole prophylaxis as standard of care for high risk neonates.

The positive catheter tips that were found Candida positive with an incidence rate of 5% might indicate that the unit or the neonates are colonized with Candida or that sterile techniques while placing central lines might not be strictly adhered to, which put the VLBW and ELBW neonates at high risk to develop invasive disease. Although only 36% that cultured Candida from their CVP tip also had invasive disease. This warrants further investigation and interventions to minimize the risk.

Initiating fluconazole prophylaxis in the high risk neonates, in other words the VLBW and ELBW neonates, will decrease the incidence rate of invasive candidemia. This will impact not only directly on morbidity associated with ICS, but also could decrease financial strains and indirectly relieve pressure on human resources.

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Appendices

Letter of approval from Research Ethics Committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

05-Jun-2019

Dear **Dr Leandri De Klerk**

Ethics Clearance: **A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the Neonatal ICU and High care unit in Universitas Academic Hospital over a 2 year period from January 2016 to December 2017 to determine if the Unit will qualify for the use of Fluconazole prophylaxis.**

Principal Investigator: **Dr Leandri De Klerk**

Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2019/0168/2506**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za

Permission from DOH

health
Department of Health
FREE STATE PROVINCE

06 May 2019

Dr L De Klerk
Dept. of Paediatrics and Child Health
UFS

Dear Dr L De Klerk

Subject: A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the Neonatal ICU and High care unit in Universitas Academic Hospital over a 2 year period from January 2016 to December 2017 to determine if the Unit will qualify for the use of Fluconazole prophylaxis.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of **Universitas Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeelats@fshealth.gov.za / lithekom@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- **Please discuss your study with Universitas Hospital CEO's on commencement for logistical arrangements see 2nd page for contact details.**
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 7/5/19

Head : Health
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Permission from NHLS

NATIONAL HEALTH LABORATORY SERVICE

Practice No. 5200296

Office of the Business Manager
UNIVERSITAS ACADEMIC LABORATORIES
PO BOX 339(G3)
C/O: CHEMICAL PATHOLOGY
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BLOCK C
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF FREE STATE
BLOEMFONTEIN
9301

REQUEST FOR APPROVAL OF LABORATORY RESOURCES FOR ACADEMIC PURPOSES

Date: 21 January 2019

Requestor: Dr. L de Klerk

Project Name: "A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the Neonatal ICU and High care unit in Universitas Academic Hospital over a 2-year period from January 2016 to December 2017 to determine if the Unit will qualify for the use of Fluconazole prophylaxis."

Dear Dr. de Klerk,

Your request for use of laboratory facilities / data is hereby granted under following conditions:

1) That University Ethical Committee approval and approval from the Universitas Hospital management is obtained
2) All laboratory data remain confidential to the patient and doctor (anonymity is maintained)
3) This Office must be notified before any publication of any results / findings are made.
4) NHLS is recognised in all publications
5) That a successful K-Project application be made and relevant NHLS project cost centre be created to utilise testing at NHLS as per your protocol.

May your project be successful.

Regards

NATIONAL HEALTH LABORATORY SERVICE
21 JAN 2019
BUSINESS MANAGER
UNIVERSITAS ACADEMIC LABORATORIES

Mr. Pakiso Letanda
Acting Business Manager

Permission from HOD

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

[redacted]

The Chair: Health Sciences Research Ethics Committee
Dr SM Le Grange
For Attention: Mrs M Marais
Block D, Room 104,
Francois Retief Building
Po Box 339 (G40)
Nelson Mandela Drive
Faculty of Health Sciences
University of the Free State
Bloemfontein
9300

21 January 2018

Dear Dr SM Le Grange

Dr Leandri de Klerk (Student number: 2007008119)
A retrospective analysis of the incidence of invasive candidiasis in extreme- and very low birth weight neonates admitted to the Neonatal ICU and High care unit in Universitas Academic Hospital over a 2 year period from January 2016 to December 2017 to determine if the Unit will qualify for the use of Fluconazole prophylaxis.

I, André Venter, hereby grant Leandri de Klerk permission to conduct the above mentioned research project. The research will be completed in accordance with myself as Head of Department of Paediatrics and Child Health and Dr Riana van Zyl as supervisor of this study.

Yours faithfully

Prof A Venter

21/1/2018
Date

Department of Paediatrics and Child Health / Departement Pediatrie en Kindergesondheid
205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
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Copy of research protocol approved by HSREC

A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the Neonatal ICU and High care unit in Universitas Academic Hospital over a 2 year period from January 2016 to December 2017 to determine if the Unit will qualify for the use of Fluconazole prophylaxis.

By

Leandri de Klerk

Protocol for a mini-dissertation submitted in fulfilment of the requirements for the degree

Master of Medicine in Paediatrics

in the

Department of Paediatrics and Child Health

Faculty of Health Sciences at the University of the Free State

CANDIDATE

Dr L de Klerk
Registrar: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State
Student number: 2007008119

SUPERVISOR

Dr R van Zyl
Consultant: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State

TABLE OF CONTENTS

LIST OF ACRONYMS

CLD:	Chronic lung disease
CMSA:	College of Medicine of South Africa
CSF:	Cerebrospinal fluid
ELBW:	Extreme low birth weight
HPCSA:	Health Professions Council of South Africa
HSREC:	Health Sciences Research Ethics Committee
ICS:	Invasive Candidiasis
NHCU:	Neonatal High Care Unit
NHLS:	National Health Laboratory Service
NICU:	Neonatal Intensive Care Unit
PVL:	Periventricular leukomalacia
ROP:	Retinopathy of prematurity
UAH :	Universitas Academic Hospital
VLBW:	Very low birth weight

SELECTED DEFINITIONS AND TERMS

Biostatistics: Data analyzed are derived from the biological sciences and medicine.

Blood/Urine/CSF culture: Blood/urine/cerebrospinal fluid specimen collected under sterile procedures and put in a culture bottle that is able to grow a species if present.

Candida bacteremia: A candida infection present in the bloodstream of an individual, proven by a blood culture that isolated the species.

Cerebrospinal fluid: Cerebrospinal fluid is a body fluid found in the brain and spinal cord. It is produced by the specialized ependymal cells in the choroid plexuses of the ventricles of the brain, and absorbed in the arachnoid granulations.

Chronic lung disease: Permanent lung damage that results in oxygen dependency.

Extreme low birth weight: Infants born with a birth weight of less than 1 kg.

Fluconazole: Anti-fungal medication used to treat fungal infections.

Incidence: Total number of new cases of a specific disease or illness reported in a specific unit in a specific time frame.

Invasive candidiasis:	A positive culture for Candida on any sterile fluid of the body, mainly urine, cerebrospinal fluid and blood.
Neonate:	Classified as the infant from birth to 28 days of life.
Periventricular leukomalacia:	Form of white matter injury in the brain, which causes neurological abnormalities.
Prematurity:	Infants born before 37 completed weeks of gestation.
Prophylaxis:	Treatment given in order to prevent a certain disease or illness.
Retinopathy of prematurity:	This is a potentially blinding disease caused by abnormal development of retinal blood vessels in the premature infants. The retina is the inner layer of the eye that receives light and turns it into visual messages that are sent to the brain.
Sepsis:	An overwhelming immune response to an infection in the bloodstream of an individual.
Very low birth weight:	Infants born with a birth weight between 1 kg and 1.5 kg.

SUMMARY

Invasive candida infection is a leading cause of mortality and morbidity during the neonatal period, specifically in infants admitted to intensive care units. Fluconazole prophylaxis has been proven to effectively reduce the incidence of invasive candida infection in neonates admitted to high care facilities.

The aim of the study is to calculate the incidence of invasive candidiasis in the extreme- and very low birth weight neonates admitted to Universitas Academic Hospital (UAH) during the years 2016 and 2017. If the incidence is high enough, this unit will qualify for the use of Fluconazole prophylaxis. The results from the research can hopefully be utilized to change current protocols.

If the incidence of invasive candidiasis can be reduced, the morbidity that walks hand in hand with this form of sepsis will also be reduced leaving these babies with a better quality of life.

This research study will be a retrospective, quantitative, non-experimental study. A literature study will be done to evaluate the current research available to support the importance of this study and to highlight the gaps in knowledge that needs to be addressed. This protocol contains the intended research study to be conducted; including the proposed research design and methodology

1. INTRODUCTION TO PROTOCOL

In this research project the incidence of invasive candidiasis in the extreme-(ELBW) and very low birth weight (VLBW) infants admitted to Neonatal ICU and HCU of Universitas Academic Hospital (UAH) from January 2016 to December 2017 will be calculated. The aim is to evaluate if this Unit qualifies for the use of Fluconazole prophylaxis. I will refer to Neonatal ICU and HCU combined as the Neonatal Unit of UAH. The main focus of this study will be to determine the total number of positive Candida cultures in the Neonatal Unit.

Incidence refers to the total number of new cases reported for a specific disease during a

specific time period. Sepsis is defined as the presence of bacteria in the bloodstream which spreads throughout the body. Invasive candidiasis is defined as a fungal sepsis that occurs in hospital settings, usually after a prolonged stay in a ward where the ward or the patients get colonised. Fungal sepsis carries a high mortality and morbidity risk for neonates (1). This necessitates the investigation of possible preventative measures – including Fluconazole prophylaxis.

2. BACKGROUND TO THE RESEARCH PROBLEM AND LITERATURE REVIEW

Neonatal sepsis remains one of the leading causes of death in the Neonatal Unit. (1) The premature infant has the highest risk of developing sepsis. Other risk factors that have been associated with an increased risk to develop neonatal sepsis include the use of total parental nutrition, mechanical ventilation, use of broad spectrum antibiotics, gastrointestinal surgery and the insertion of central venous lines. (2, 3, 6) The mortality caused by fungal sepsis in Neonatal Units ranges 25-50%. (1, 8)

The premature infant is largely at risk for the development of invasive candidiasis (ICS), due to their immature immune system. (4, 5) The complications associated with ICS include shock, meningitis and renal failure. After surviving ICS the neonates are at higher risk of developing retinopathy of prematurity (ROP), periventricular leukomalacia (PVL) and chronic lung disease (CLD). (7) Infants who had ICS compared to the infants who did not have ICS showed a global delay in neurological development at the age of 18 months. (21)

Fungal sepsis usually is a cause of late onset neonatal sepsis and carries a high mortality and morbidity risk. (8, 1) After being diagnosed with invasive candidiasis, the treatment with intravenous anti-fungal therapy is usually a minimum of 14 – 21 days. (16) This leads to a prolonged hospital stay and increased cost to the patient and/or hospital.

Research has been done that suggest that if the incidence of Fungal sepsis in a neonatal unit is higher than 5%, the use of Fluconazole prophylaxis at a dosage of 3-6mg/kg twice weekly

for a total period of three weeks can decrease the incidence of invasive candidiasis in that unit. (9-13, 15) The incidence of invasive candida ranges between 2-4% and can go up to 16% in extreme low birth weight infants (<1000g). There is little evidence that shows that Fluconazole prophylaxis has an effect on the reduction in mortality rate caused by fungal sepsis, but the morbidity caused by the invasive sepsis is decreased. (9, 15)

If one can decrease the incidence of fungal sepsis in the Unit, then the Unit will be less colonized with Candida and secondary to that the overall sepsis risk of the ward will decrease. (14) There is a link between ICS and bacterial sepsis; it was documented that a decrease in ICS also reduces the bacterial sepsis rate. (17, 18) By decreasing the overall sepsis in the Unit, the length of stay and length of usage of antibiotics will be decreased. This has clear financial implications for the healthcare system.

No immediate side effects were reported or found in the studies where Fluconazole prophylaxis was given to the patients; the drug was reported as safe for usage as prophylaxis in the neonate. (22) Fluconazole prophylaxis had no effect on liver function tests or the resistance profile of the Candida species. Fluconazole prophylaxis that is used for more than four weeks may have an influence on the resistance profile of the Candida species.(19) Follow up of the patients who received four week treatment with Fluconazole prophylaxis did not show any side effects on the neurological development of the patients.(20)

3. PROBLEM STATEMENT

The purpose of the proposed study is to determine the incidence of invasive candidiasis in the Neonatal Unit of Universitas Academic Hospital (UAH). The study will focus on the positive blood cultures for candida species during the years 2016 and 2017 in the ELBW and VLBW infants admitted to this specific unit.

The high sepsis rate at the Neonatal Unit at UAH is a big concern and contributes to increased mortalities. Challenges such as staff shortages, overcrowding and lack of consumables increase the number of sepsis cases in this unit. According to the monthly

statistics that are presented at UAH, rates of sepsis are high and several organisms are cultured in the unit. So far, there have been no specific incidence rates calculated to proof that invasive candidiasis is a major challenge in this Unit.

The incidence of invasive candidiasis according to literature is 2–4 %. If the incidence is higher than 5-10%, the use of Fluconazole prophylaxis in the unit can be helpful to decrease the incidence of invasive candidiasis. (15) If it is lower than 5%, the patients' risk factors should be weighed up individually to determine if prophylaxis will be helpful.

4. AIM OF THE STUDY

The aim of the study is to calculate the incidence of ICS in the neonatal ICU and HCU of UAH in order to determine whether the unit qualifies for the use of Fluconazole prophylaxis. The Fluconazole prophylaxis will not be implemented in this study, this study will only calculate the incidence of ICS in the units.

5. RESEARCH QUESTIONS

What is the incidence of invasive candidiasis in the ELBW and VLBW infants admitted to Neonatal ICU and HCU at UAH for the year 2016 & 2017?

Is the incidence high enough (>5%) for the unit to qualify for Fluconazole prophylaxis?

6. OBJECTIVES OF THE STUDY

Primary objective: Determine the incidence of invasive candidiasis in the ELBW and VLBW neonates admitted to the Neonatal Unit of Universitas Academic Hospital from January 2016 to December 2017.

Secondary objective: Compare the incidence as mentioned in the primary objective to literature and determine if the unit qualifies for the use of Fluconazole prophylaxis.

7. RESEARCH DESIGN

Study design:

The study design chosen for this particular research is a retrospective descriptive cross sectional study. This study design will enable the researcher to compare the positive invasive candidiasis results against the negative invasive candidiasis results. Drawing up a two by two table will also group the birth weight category of the infants in extreme low birth weight and very low birth weight that is defined as lower than 1.5kg. This study will measure quantitative results. The table will also show what type of body fluid was tested positive for Candida, the gender of the baby, gestational age at birth and age at the time of the positive culture results.

Target population and population size:

All infants that has a birth weight below 1.5kg and admitted to Universitas Neonatal ICU and HCU from 1st January 2016 to 31st December 2017 will be included in the study. This usually ranges between 20 to 40 per month according to the statistics presented monthly. Total sample size will be more or less 500 to 900 babies who would have been admitted during the study period. The patient's that demised will not be excluded from the study.

Data sources:

Data will be collected from the following databases: Meditech, NHLS and Department Microbiology records. NHLS and Microbiology will provide the positive cultures for Candida species in the Neonatal Unit for the years 2016 and 2017. After collecting all the positive blood culture results, the profile of the patients can be determined using the hospital number on Meditech to identify the weight category, gender and age at onset of the infection. If Meditech does not have the data of the patient, the original file of the patient will be collected. All the infants with a birth weight below 1.5kg will be documented even if they have a negative culture, in order to work out the incidence both positive and negative results need to be captured.

The biostatistician has approved the data sheet that will be used to capture patient data.
To prevent bias the research must be done focused on the research question and not to gather information not needed to answer the research question. This study can also be compared with similar studies to test the validity of the study.

The confidentiality of patient information will be prioritized. The files and the data capture sheets will only be managed by the researcher in a private room. The patients will also get a number allocated to them to protect their identity.

Pilot study:

A pilot study will be conducted on five neonates who fit the inclusion criteria of the research project. The pilot study will assess the effectiveness of the data capture sheet and if necessary amendments can be made. These results will be included in the final study.

7.1 Data analysis and interpretation

This will be done by the help of a qualified biostatistician employed by the University of the Free State.

8. ETHICAL CONSIDERATIONS

8.1 Approval

Approval to conduct the research will be obtained from the Health Sciences Research Ethics Committee at the University of the Free State (HSREC), Free State Department of Health (DOH), National Health Laboratory Services (NHLS) and the Head of Department of Microbiology. No data collection will commence prior to final approval from the HSREC has been obtained.

8.2 Informed consent

No informed consent is necessary from participants because the researcher will not be working with the patients directly or participating in any procedures. This study only consists of retrospective collection of data that has already been stored on the databases and

electronic patient records.

8.3 Right to privacy and confidentiality

The researcher will sit in a private room when accessing the data of the patients. The names and hospital numbers will be replaced by unique patient identifiers to protect the privacy of the patient. The researcher will not share the patients' personal details with any other person. All data sent for analysis will be unidentifiable to the biostatistician. All information will be managed in a strictly professional and confidential manner.

9. TIME SCHEDULE

Time schedule for executing the research

Preliminary literature study	*January 2018 – April 2018*
Protocol writing	*May 2018 – November 2018*
Evaluation Committee	*November 2018*
Submission to HSREC	*February 2019*
FS DOH approval	*February 2019 – April 2019*
Final Ethics approval	*May 2019*
Data collection	*June– July 2019*
Data and statistical analysis	*August 2019*
Report writing	*September – November 2019*
Report submission	*November 2019*

10. BUDGET

Estimated budget for executing the study

PLANNED ITEM	COST PER ITEM	QUANTITY	TOTAL AMOUNT
Photocopies	R1.00	500	R500.00
Stationary(pens/highlighters)	R10.00	10	R100.00
Data bundles	R0.10	5000	R500.00
TOTAL			R 1100.00

Application for funding will be made from the following:

- Postgraduate School of the University of the Free State

11. REFERENCES

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Forms for collecting data

Appendix :

Data capture sheet:

Neonatal information:	
Infant case number	
Date of birth	d m y
Date of admission	d m y
Age at onset of invasive candideamia	days
Gender	Female=0 Male=1
Gestational age	Term=0 Preterm=1
Birth weight	g
Candida Blood culture positive	No=0 Yes=1
Candida Urine culture positive	No=0 Yes=1
Candida CSF culture positive	No=0 Yes=1
Death	No=0 Yes=1

F

AOSIS

APPENDIX J: INSTRUCTIONS TO AUTHORS – SAJHIVMED (AOSIS)

https://sajhivmed.org.za/index.php/hivmed/pages/view/submission-guidelines#part_1

Original Research Article full structure

_____

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of five paragraphs labelled Background, Objectives, Method, Results and Conclusion.

- Background: Why do we care about the problem? State the context and purpose of the study. (What practical, scientific or theoretical gap is your research filling?)

- Objectives: What problem are you trying to solve? What is the scope of your work (e.g. is it a generalised approach or for a specific situation)? Be careful not to use too much jargon.

- Method: How did you go about solving or making progress on the problem? State how the study was performed and which statistical tests were used. (What did you actually do to get the results?) Clearly express the basic design of the study; name or briefly describe the basic methodology used without going into excessive detail. Be sure to indicate the key techniques used.

- Results: What is the answer? Present the main findings (that is, as a result of completing the procedure or study, state what you have learnt, invented or created).

Identify trends, relative change or differences on answers to questions.

- Conclusion: What are the implications of your answer? Briefly summarise any potential implications. (What are the larger implications of your findings, especially for the problem or gap identified in your motivation?)

Do not cite references and do not use abbreviations excessively in the abstract.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

- Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by use of evidence from the literature.

- Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic, and should clarify the knowledge gap that this study will address. Your argument should be supported by use of evidence from the literature.

- Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.

- Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design: This must address the following:

- Study design: An outline of the type of study design

- Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.

- Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.

- Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.

- Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.

- Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used orsteps followed in qualitative data analysis.

- Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.

Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the SI convention and be abbreviated accordingly.

Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma

Discussion: The discussion section should address the following four elements:

- Key findings: Summarise the key findings without reiterating details of the results.

- Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.

- Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.

- Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.
Also provide the following, each under their own heading:

- Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article. Read our policy on competing interests.

- Author contributions: All authors must meet the criteria for authorship as outlined in the authorship policy and author contribution statement policies.
- Funding: Provide information on funding if relevant

- Disclaimer: A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

References: Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Refer to the journal referencing style downloadable on our Formatting Requirements page.

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Abstract

Background: Invasive Candida infection is a leading cause of mortality and morbidity during the neonatal period, specifically in premature neonates admitted to intensive care units. Fluconazole prophylaxis has been proven to effectively reduce the incidence of invasive candidiasis in neonates admitted to high care facilities.

Methods: A retrospective, descriptive, cross-sectional, non-experimental study was conducted. Data was collected from the MediTech and National Health Laboratory Service databases. Positive culture results on sterile body fluids were analyzed to determine the incidence of invasive candidemia in the neonatal unit at Universitas Academic Hospital. Sterile body fluids included blood, urine and cerebrospinal fluid.

Results: The results revealed an incidence of 13.5% of invasive candidiasis in the extreme and very low birth weight neonates admitted to the neonatal unit at Universitas Academic Hospital. These results included all the positive cultures done on urine, cerebrospinal fluid and blood. The study population was 324 neonates admitted to the neonatal unit of Universitas Academic Hospital with a birth weight of less than 1.5 kg for the years 2016 and 2017. The population group included 183 female neonates (56%) and 141 male neonates (44%). The median gestational age was 29 weeks and the median birth weight was 1110 g. The positive Candida culture results included 43 blood cultures, 5 urine cultures and zero cerebrospinal fluid cultures.

Conclusion: The incidence rate for invasive candidiasis in very low birth weight and extreme low birth weight neonates at Universitas Academic Hospital was 13.5% during the study period. Previous research studies concluded that an incidence rate higher than ten percent of invasive Candida infection is significant and a neonatal unit will benefit from the use of fluconazole prophylaxis. Decreasing the incidence will have a direct effect on the mortality and morbidity associated with neonatal invasive Candida infection. The recommendation following the findings of the study would be to include the use of fluconazole prophylaxis as standard of care in the neonatal unit at

A retrospective analysis of the incidence of invasive candidiasis in the extreme - and very low birth weight neonates admitted to the neonatal ICU and high care unit in Universitas Academic Hospital o

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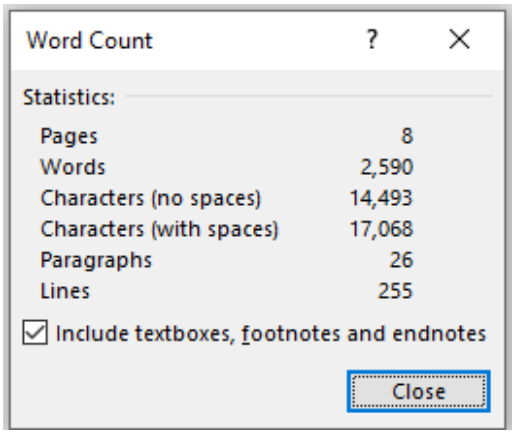

Chapter 2