CAUSATIVE INFECTIONS IN CHILDHOOD CANCER PATIENTS WITH FEBRILE NEUTROPENIA IN PIETERSBURG HOSPITAL, LIMPOPO PROVINCE, SOUTH AFRICA

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N.E MOTHIBA

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CAUSATIVE INFECTIONS IN CHILDHOOD CANCER PATIENTS WITH FEBRILE NEUTROPENIA IN PIETERSBURG HOSPITAL, LIMPOPO PROVINCE, SOUTH AFRICA

by

NOMSA EDITH MOTHIBA

(MINI-) DISSERTATION

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CO-SUPERVISOR: Prof. M Kruger
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DECLARATION

I, Nomsa Edith Mothiba declare that CAUSATIVE INFECTIONS IN CHILDHOOD CANCER PATIENTS WITH CHEMOTHERAPY RELATED FEBRILE NEUTROPENIA IN PIETERSBURG HOSPITAL, LIMPOPO PROVINCE, SOUTH AFRICA hereby submitted to the University of Limpopo, for the degree Master Of Medicine In Paediatrics And Child Health has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Mothiba NE DR 05/10/2022

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ABSTRACT

BACKGROUND

Febrile neutropenia is a medical emergency that complicates the clinical course and treatment of both hematological and solid malignancies, potentially worsening the overall outcome and increasing the financial burden. The epidemiology of pathogens is varied, and determines the selection of empiric antibiotic therapy for febrile neutropenia. Empirically piperacillin/tazobactam plus amikacin has been recommended as the most suitable antibiotic for management of febrile neutropenia. There is a lack of local studies to provide advice for antibiotic choice in our setting.

OBJECTIVE

To identify causative organisms of infection and antibiotic susceptibility patterns in childhood cancer patients with chemotherapy related febrile neutropenia in Pietersburg Hospital Oncology Ward Limpopo Province.

METHODS

This is a retrospective cross-sectional study that reviewed all the febrile neutropenic episodes in children with cancer and with a positive blood culture during the febrile neutropenia episode. Data collected included patient demographics (date of birth, sex, date of diagnosis) diagnosis, organisms cultured and the antibiotic sensitivity profile.

RESULTS

There were 152 records of positive blood cultures identified of 348 episodes of febrile neutropenia for 413 patients. The median age of study population is 6years (mean age of 6.8years; range 3 to 11years) with male predominance at (61.2%). The most common cancer diagnosis was Acute Lymphoblastic Leukemia (ALL) (33.6%) followed by Nephroblastoma (15.8%), Acute myeloid leukemia (11.2%), Non-Hodgkin's lymphoma (9.9%), Hodgkin's lymphoma (5.9%) and other cancers

(15.3%). The majority of causative organisms were gram-positive bacteria (45%) followed by gram-negative bacteria (32.4%) and fungi (6.1%). Gram-positive organisms were statistically significant pathogens causing bacteraemia more often in neutropenic patients than gram-negative organisms with a p value=0.016. The majority (*n*=102; 67.10%) were sensitive organisms with the minority being multidrug resistant organisms (*n*=23; 15.1%) and 17.8% were contaminants *n*=27. The most common gram-positive pathogens were *Coagulase negative staphylococcus n=37*; (21.6%). The most common multidrug resistant organisms were *Klebsiella pneumoniae CRE* (10.7 %;), followed by *Enterococcus faecium VRE* (1.9%), *Klebsiella oxytoca CRE* (1.3%), *Enterococcus faecalis VRE* (0.6%), and *Staphylococcus aureus MRSA* (0.6%). No multidrug resistant fungal organisms were cultured. The majority of organisms were sensitive to the first line empiric therapy piperacillin/tazobactam plus Amikacin (67.10%). Thirty patients died during these febrile neutropenic episodes and case fatality rate was 8.6%.

CONCLUSION

This study confirmed that the causative bacteria of febrile neutropenia in this study were susceptible to the first line empiric therapy piperacillin/tazobactam plus amikacin, and this regimen is therefore appropriate for this paediatric oncology unit.

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DEFINITION OF CONCEPTS

Antibiotics: prescribed drugs obtained from a microorganism that destroys, inhibits or slows progression of microbial growth (Mahon and Lehman, 2018). For this study, antibiotics are indicated to treat infections caused by microorganisms that are sensitive to them.

Bacteremia: the presence of a microorganism capable of causing an infection in the blood (Mahon and Lehman, 2018). For this study, this means the presence of microorganisms causing a bloodstream infection.

Broad spectrum antibiotics: antibiotics active against a vast variety of microorganisms (Mahon and Lehman, 2018). For this study, this means antibiotics used to cover a wide variety of microorganisms cultured from blood.

Chemotherapy related febrile neutropenia: a clinical condition relating to fever in the presence of neutropenia secondary to chemotherapy induced immune dysfunction (Lanzkowsky, Jeffry and Jonathan, 2016). In this study it will refer to those who develop fever and neutropenia after receiving chemotherapy.

Chemotherapy/cytotoxic therapy: the prevention or treatment of malignancy by the use of chemical substances to inhibit abnormal cell growth (Robins and Kumar, 2017). In this study it will refer to therapy used to treat malignancies.

Contaminants: a false positive blood culture result which is not of clinical consequence occurring when pathogens that are not present in a blood specimen are grown in culture (Mahon and Lehman, 2018).

Empirical antibiotics: antibiotic therapy which refers to the use of antibiotics to treat a suspected infection before the identification of the organism and its antibiotic susceptibility profile (Mahon and Lehman, 2018). For this study, this means the use of antibiotics without prior knowledge of the microorganisms causing infection and its antibiotic susceptibility pattern.

Febrile: A state relating to a rise in body temperature above normal (Lanzkowsky, Jeffry and Jonathan, 2016). In this study, it will be defined as a solitary axillary temperature of more or equivalent to 38.5^{o}C or temperature of 38^{o}C that occurs twice within a 24hour period.

Febrile neutropenia: a clinical condition relating to a rise in body temperature of more than 38ºC with an absolute neutrophil count of less than 500cells/microliter secondary to the malignancy or the presence of an identified source of infection (NICE guidelines, 2012). For this study, it means a rise in body temperature and neutropenia secondary to the presence of an identified source of infection.

Haematological disease: refers to a diseased state affecting blood or blood producing organs (Lanzkowsky, Jeffry and Jonathan, 2016). For this study, it means any pathological condition affecting blood or blood producing organs.

Malignancy: disease state characterized by abnormal cell proliferation which is disorganized with that of normal tissues and can invade adjacent structures or spread to distant sites (Robins and Kumar, 2017). For this study, only those with proven histological evidence will be referred to as having a malignancy.

Morbidity: a diseased state or any physical or psychological state considered to be outside the realm of normal well-being (Stedman Medical Dictionary, 2005). In this study it will refer to a diseased state as a result of complications arising from infection.

Mortality: a quantity of deaths in a certain population at a specific time period (Kibel and Wagstaff, 2001). In this study it will refer to the number of patients who demised as a result of infection.

Neutropenia: A decrease in the number of neutrophil count defined as an absolute neutrophil count of less than 1500cells/mm^3 (Pizzo and Poplack, 2015). In this study it will be defined as an absolute count of less or equal to 500cells/mm^3.

Neutropenic sepsis: a life-threatening complication of anticancer treatment used to describe a significant inflammatory response to a presumed bacterial infection in a person with a temperature of more than 38ºC with an absolute neutrophil count (ANC) of less than 500 cells/microlitre (NICE guidelines, 2012). For this study, this means an overwhelming infection in a person with a low neutrophil count.

LIST OF ABBREVIATIONS

ALL: Acute lymphoblastic leukemia

AML: Acute myeloid leukemia

ANC: Absolute Neutrophil Count

BMI: Body Mass Index

CRP: C-reactive protein

ESBL: Extended spectrum beta lactamase

FN: Febrile Neutropenia

HIV: Human Immunodeficiency Virus

IATG: International Antimicrobial Therapy Group

ICU: Intensive Care Unit

IDSA: Infectious Disease Society of America

NHLS: National Health Laboratory Services

NICE: National Institute for Health and Care Excellence

POU: Paediatric Oncology Unit

TB: Tuberculosis

WHO: World Health Organization

CHAPTER ONE

1. BACKGROUND AND RESEARCH PROBLEM

1.1Introduction and background

Febrile neutropenia is a clinical condition relating to a rise in body temperature in the presence of neutropenia secondary to the malignancy itself or induced by chemotherapy. This condition remains a common complication among patients with hematological and solid cancers undergoing various cytotoxic therapies. Despite major improvement in antibiotic combinations used as empirical therapy for febrile neutropenia the condition is associated with substantial morbidity and mortality (Perron, Erama and Ahmed, 2014). Febrile neutropenia may also impede the administration of chemotherapy, prolonged hospital stay and result in additional financial costs associated with directed microbial therapy and overall high mortality rates especially when complicated with an overwhelming neutropenic sepsis (Karimi, Farzaneh, Azadeh and Ali, 2018).

The epidemiology of pathogens is dynamic, differs across various settings and determines the selection of empiric antibiotic therapy for febrile neutropenia. It is therefore extremely important to investigate the causative pathogens of nosocomial sepsis to facilitate therapeutic decision making (Caselli and Olivier, 2015). Surveillance data from the International Antimicrobial Therapy Group (IATG) of the European organization for research and treatment of cancer demonstrated a change in the aetiology of infection and in the patterns of resistance. Before the mid-1980s gram-negative bacilli were the predominant pathogens causing 60-70% of bacteremia in neutropenia patients, but since the late 1980s it changed to predominantly gram-positive cocci (Giulia and Malgorzata, 2016). This shift had enormous implications for the care of these patients.

A multicenter design study in Netherlands and Switzerland analyzed positive blood cultures of paediatric cancer patients presenting with febrile neutropenia and showed

a predominance of gram-positive bacteria in patients with febrile neutropenia (Agyeman, Kontny, Nadal and Leibundgut, 2014). Gram-negative bacteraemia due to Pseudomonas aeruginosa, were predominantly in Switzerland, where Ciprofloxacin prophylaxis was not routinely used as a prophylactic agent. Resistance to Ciprofloxacin was reported commonly in those who received prophylactic Ciprofloxacin. The antibiotic susceptibility profile might differ from one region to the other due to differences in geographical factors influencing the risk to the acquisition of antibiotic resistance. Empiric antibiotic therapy should be based on the locally prevalent microorganisms and antibiotic profile (Miedema, Winter, Amman and Droz, 2013). A single center South African study found that the causative organisms of febrile neutropenia over a two year study period were mainly due to gram-positive organisms (58%) (Green and Kruger,2016).

Management of febrile neutropenia involves hospitalization and administration of intravenous broad-spectrum antibiotics to provide empirical cover for both gram-positive, as well as gram-negative bacteria (Perron et al, 2014). The approach for empiric antimicrobial therapy for febrile neutropenia should be guided by commonly cultured pathogens and antibiotic sensitivity patterns per local setting (Green and Kruger, 2016).

The proposed research investigates the profile of causative organisms of febrile neutropenia among patients with cancer receiving chemotherapy as well as their antibiotic sensitivity patterns in a paediatric oncology unit in Limpopo province, South Africa.

1.2 Research problem

Chemotherapy related febrile neutropenia is a common complication in our setting and patients tend to have poor clinical outcomes despite instituting internationally recommended treatment guidelines.

Studies have reported that Piperacillin/tazobactam plus amikacin have been recommended as the most suitable empirical therapy to cover most bacteraemia causing pathogens (Aisha, Naveena, Sajjad, Bushra, Qurratul, Uzma, Munira and Tahir, 2019) in Iran & (Naima, Saad, Sittana and Mohammed, 2014) in Qatar, Western Asia. The lack of local studies that have analyzed local data to inform antibiotic choice in the South African setting has prompted the need for more research and to design local treatment protocols using local data.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Incidence of childhood cancer

The overall age-standardised rate (ASR) of cancer in children aged 0-14years was 140.6 per million person-years and 155.8 per million person-years in those aged 0-19years(Steliarova-Foucher, Colombet, Ries and Moreno,2017).Childhood malignancy remains the second most common cause of death in children aged 5-14 years in high-income countries, contributing up to 8% of the global post neonatal mortality rate according to a report by the World Health Organization (WHO) in 2015 (Bhakta, Force, Allemani and Atun,2019). The incidence of childhood cancer in South Africa is reported as 45 per million children per year presumably (Steliarova-Foucher et al, 2017).

2.2 Mortality rates of childhood cancer patients with febrile neutropenia

The five-year overall survival for childhood cancer is currently close to 80% in high-income countries due to enormous improvements in supportive care, but in spite of the enormous improvements in supportive care this is not yet the case for low-and middle-income countries (Steliarova-Foucher et al, 2017). Nearly 16% of deaths within five years of diagnosis are the result of treatment complications, including febrile neutropenia (Steliarova, Fidler, Colombet and Lacour, 2018). Close to 50% of deaths in patients receiving chemotherapy for solid tumours can be due to febrile neutropenia while it can be between 50% and 75% for children with acute leukemia (Rasmy, Amal, Fotih and Selwi, 2016). In the USA, a death rate associated with neutropenia ranges from 3.4 % to 10.5% with general cancer mortality between 6.8% and 9.5% (Ozguler, 2015).

A recent South African study from a single institution reported a case fatality risk due to severe sepsis to be 80%, especially due to prolonged neutropenia (Naidu, Izu, Poyiadjis and MacKinnon, 2020). However, there was no association between malnutrition or HIV infection and death.

2.3 Diagnosis of chemotherapy related febrile neutropenia and neutropenic sepsis

Febrile neutropenia is identified clinically with the development of fever in the presence of neutropenia (Lustberg, 2012). The evaluation starts with a thorough history and examination of common sites of infection followed by laboratory analysis which include a full blood count with differential count and other supportive acute phase reactants which include a C-reactive protein (CRP) and Procalcitonin, although their use has a poor predictive value for neutropenic sepsis (Lustberg, 2016).

A positive blood culture should be obtained from a sterile site namely blood, cerebrospinal fluid or urine. Culture results are often delayed and available only after 48-72hours; therefore, initiation of empirical antibiotics relies on good clinical judgment and knowledge of local causative organisms (Lustberg, 2012). It is imperative to initiate treatment as soon as blood cultures are obtained. Blood cultures should be obtained without delay. Blood cultures must be withdrawn from every lumen of the central line when such access is available. Although peripheral cultures increase identification of true bacteraemia compared with CVC cultures alone, the impact of increased yield is unknown (Kebudi and Kizilocak, 2018).

CSF examination and CSF cultures are recommended when meningitis is suspected, and urine cultures recommended when a clean catch specimen can be obtained. While routine chest x-rays are recommended for those patients with respiratory signs and symptoms or clinical deterioration on antibiotics for duration of four to seven days (Kebudi and Kizilocak, 2018).

2.4 Pathogenic organisms implicated in neutropenic sepsis

In the 1960s and 1970s the incidence of gram-negative infections was reported more commonly but this changed due to the increased use of indwelling catheters, early-generation quinolone prophylaxis, and broad-spectrum empirical gram-negative antibacterial therapy which led to an increase in the incidence of gram-positive

pathogens in the 1980s and 1990s (Klaassen, Goodman, Pham & Doyle, 2016). These infections were a major cause of poor prognosis and death in paediatric cancer patients (Klaassen et al, 2016).

The commonly cultured gram-positive pathogens were *Coagulase-positive staphylococci,* especially *Staphylococcus* aureus including methicillin-resistant *S. aureus* and *Streptococci* (Hann, Viscoli, Paesmans, Gaya and Glauser, 2017). Aerobic gram-negative bacilli are likely to cause approximately one-third to one-half of bacteraemic episodes, with *Escherichia coli, Klebsiella, Pseudomonas, Acinetobacter* and *Enterobacter* species among the more common isolates (Hann et al, 2017).

Fungal, pathogens typically *Candida* species are more likely to be recovered after prolonged courses of broad-spectrum antibiotics but occasionally may be the primary pathogen (Freifeld, Bow, Sepkowitz, Boeck and Ito, 2016). Other potential fungal organisms include *Aspergillus, Zygomycetes* and *Cryptococcus* (Wisplinghoff, Seifert, Wenzel and Edmond, 2003). The increasing use of antifungal prophylaxis is likely to shift the distribution of fungal isolates away from candida species towards mold infections. The most significant viral causes are herpes simplex and varicella-zoster virus; respiratory viruses are also frequently detected in nasopharyngeal aspirates (Hakim, Flynn, Knapp, Srivastava and Gaur, 2019).

2.5 Risk factors and risk stratification for morbidity and mortality in febrile neutropenia

Patients with febrile neutropenia can be stratified as high and low risk for severe infection or complications based upon presenting signs and symptoms, absolute neutrophil count (ANC), underlying cancer, anticipated duration of neutropenia and medical comorbidities. High risks are classified as those who develop neutropenia with ANC <500 cells/microlitre anticipated to last more than 7 days with associated comorbidities, evidence of multiorgan insufficiency, infants with ALL (acute lymphoblastic leukemia) and those with AML (acute myeloid leukemia). Low risks are those who develop neutropenia expected to resolve within seven days, with no other associated comorbidities (Freifeld et al, 2016).

Risk factors identified in low-to-middle-Income countries (LMICs) were the burden of HIV (human immunodeficiency virus), TB (Tuberculosis) and malnutrition as associated with poor survival in children who develop chemotherapy related febrile neutropenia (Ammann et al, 2010). Children from low-to-middle-income countries often suffer from malnutrition when a malignancy is diagnosed. Malnutrition in paediatric cancer patients makes them prone to chemotherapy-related toxicity and infections, and increases the risk of death among this population.

In both adult and paediatric studies looking at the risk of HIV and febrile neutropenia, advanced HIV disease characterized by a low CD4 cell count was linked with the development of neutropenic fever and prolonged neutropenia during chemotherapy (Stefan et al, 2015). In a South African adult study, HIV infection was shown to be an independent risk predictor of chemotherapy induced neutropenia, this was due to neutropenia and neutrophil dysfunction associated with HIV infection (Ngidi, Magula, Sartorius and Govender, 2017).

2.6 Recommended empirical antimicrobial regimens for treatment of febrile neutropenia and neutropenic sepsis

The cornerstone of treatment in these patients is initiation of empiric broad-spectrum antibiotics (Katsimpardi, Papadakis, Pangalis, Parcharidou and Panagitou, 2016). Delay of more than 60 minutes after presentation has been associated with poor outcomes and lengthy hospital stay. Recommendations have been published for the indication of empiric antibiotic use during episodes of febrile neutropenia, including those recommended by the Infectious Diseases Society of America (IDSA), most recently updated (Melgar, Reljic, Barahona and Camacho, 2020). These include the International Pediatric Fever and Neutropenia Guideline Panel (2017), and the National Institute for Health and Care Excellence (NICE) (Melgar et al, 2020). The use of broad-spectrum empiric antibiotics for febrile neutropenia in both adult and paediatric studies is well established because it has been found that 10 to 24% of these patients are bacteraemic (Katsimpardi et al, 2016). Empiric antifungal therapy is not recommended for routine initial therapy in low-risk patients (AMJC guidelines, 2017).

Patients with FN stratified as high risk for adverse effects should be started on empiric antibiotics administered intravenously in the in-patient hospital setting until fever subsides and ANC improves to >500/mm3 (Kebudi and Kizilocak, 2018). Empiric antibiotic therapy should have a wide spectrum covering gram-positive and gram-negative bacteria, including antipseudomonal cover (Kebudi and Kizilocak, 2018). Initial treatment may include monotherapy with antipseudomonal penicillin or combination therapy (addition of aminoglycoside or glycopeptide) in patients who are clinically unstable or when a resistant infection is suspected (Kebudi and Kizilocak, 2018).

Studies comparing monotherapy and combination therapy have proven monotherapy to be as effective as combination therapy and less toxic (Freifeld et al, 2010). It is advised that Vancomycin should not be used empirically as part of the initial regimen or for non-resolving fever, but rather should be added when a blood culture has isolated a pathogen that requires its use (Kebudi and Kizilocak, 2018). If methicillin-resistant *Staphylococcus aureus* is likely, the initial antibiotic regimen can include vancomycin, daptomycin, or linezolid (Gea-Banacloche, 2014). The early use of carbapenem may be beneficial if an extended-spectrum beta-lactamase–producing gram-negative bacterium is suspected (Gea-Banacloche, 2014).

If the presence of *Klebsiella pneumoniae* bacteria is suspected, the addition of polymyxin-colistin or tigecycline to initial treatment is appropriate. Patients with penicillin allergy may be given cephalosporin, but either ciprofloxacin and clindamycin or aztreonam and vancomycin are recommended in cases of immediate hypersensitivity (Freifeld et al, 2010). In patients at high risk for invasive fungal infection empirical antifungal therapy should be added after 72 hours of persistent fever (Kebudi and Kizilocak, 2018).

Patients with FN stratified as low risk for complications may be initially treated with oral empirical antibiotics or intravenously in the inpatient setting. Patients with adequate gastrointestinal absorption may be eligible for de-escalation of intravenous to oral administration of antibiotics (Kebudi and Kizilocak, 2018), (Gea-Banacloche, 2014). Recommended treatment for low-risk patients includes oral fluoroquinolone

monotherapy, fluoroquinolone and amoxicillin-clavulanate, and cefixime (Kebudi and Kizilocak, 2018).

Patients who have persistent fever and deteriorating signs and symptoms of infection should remain as inpatients rather than being discharged. Initiation of empiric antifungal therapy is advised for patients who have persistent fever after 4 to 7 days of antibiotic treatment, and who present with neutropenia that is expected to last more than 7 days (Freifield et al, 2010), (Kebudi and Kizilocak, 2018).

Unfortunately, there is a challenge with the occurrence of antibacterial resistance in bacterial pathogens infecting febrile neutropenic patients. This further complicates management and makes choosing empirical antimicrobial therapy difficult. The World Health Organization (WHO) has identified antimicrobial resistance as one of the three major threats to human health which may contribute to increased rates of morbidity and mortality (El-Mahallawy, Mohamed, Manar, 2017).

2.7 Recommended duration of antimicrobial therapy

The total duration of empiric antimicrobial therapy depends upon the clinical response and myeloid recovery (Kebudi and Kizilocak, 2018). Continued use of antimicrobial therapy until blood cultures have been negative for more than 48 hours is recommended, and treatment is necessary until the patient is afebrile for at least 48 hours, clinically stable with resolution of neutropenia (ANC of at least 500 cells per microliter) and increasing neutrophil count (Freifield et al, 2010).

CHAPTER THREE

3. PURPOSE OF THE STUDY

3.1 Study aim

To evaluate the causative organisms of infection in childhood cancer patients with chemotherapy related febrile neutropenia in Pietersburg Hospital Oncology Ward Limpopo Province

3.2 Study objectives

3.1.1 To identify causative organisms of infection in childhood cancer patients with chemotherapy related febrile neutropenia in Pietersburg Hospital Oncology Ward Limpopo Province
3.1.2 To determine antibiotic sensitivity profile among the positive blood cultures
3.1.3 To determine the outcome of these febrile neutropenic episodes

3.3 Research methodology

3.3.1 Study design:

This was a retrospective cross-sectional study involving a review of patient records seen at Pietersburg hospital oncology unit during a period of five years (01st January 2016 to 31st December 2020).

3.3.2 Study setting:

The study was conducted in the Paediatric Oncology Unit (POU) at Pietersburg hospital, a 29-bed unit at a tertiary referral hospital in Limpopo province. It is about 5 km north of Polokwane central business district, located in the Capricorn district of Limpopo Province. The unit offers tertiary hospital services in various medical disciplines including Paediatric diagnostic services for childhood cancer patients with hematological and solid cancers and provides chemotherapy services. The

Paediatric department accepts patients from its local catchment area and the surrounding communities, as well as referrals for specialist care from district and regional hospitals.

3.3.3 Study population and sample:

The study population included all children with chemotherapy related febrile neutropenia at Pietersburg hospital during the study period. The study sample included the clinical records of cases with positive blood cultures between 01st January 2016 to 31st December 2020.

3.3.4 Inclusion criteria:

- All positive blood cultures with either a bacterial or fungal pathogen from at least one blood culture obtained from a peripheral vein or central venous catheter in the presence of febrile neutropenia defined as a temperature of more or equal to 38ºC with an absolute neutrophil count (ANC) of less than 500 cells/mm^3 (NICE guidelines, 2012).
- Patients who have received chemotherapy for up to three months prior to the episode of febrile neutropenia

3.3.5 Exclusion criteria:

Records of patients with:

- Normal or absolute neutrophil count of above 500cells/mm^3
- No documented receipt of chemotherapy
- Presentation beyond the study time period

3.4 Data collection

Clinical records were used as a secondary source of data. Microbiological results were obtained from the National Health Laboratory Services (NHLS) database.

Records were obtained from the unit registry kept in the oncology ward. Data was collected using a self-developed data collection sheet which indicates all the required relevant information.

Data collection sheet consists of three sections as discussed below:

Section A: Patient related parameters

- Study number
- Date of admission
- Date of birth
- Sex

Section B: Disease related parameters

- Type of malignancy (histological diagnosis)
- Stage of malignancy
- Date of last chemotherapy administration

Section C: Laboratory parameters

- Blood culture results(positive/negative)
- Profile of organisms
- Antibiotic susceptibility pattern

3.5 Microbiological procedure

Microbiological testing of blood culture specimens was completed at the Polokwane Hospital NHLS microbiology laboratory using the BACTEC 9240 automated blood culture system. If isolation of any organism was detected, standard biochemical, disc diffusion, and gradient diffusion antibiotic susceptibility profiling were used to assess gram-positive organisms. Gram-negative organisms were isolated and tested for antibiotic susceptibility profile after direct inoculation of the bacterial colonies into the automated Vitek 2 system (bioMérieux, Inc., France) using Vitek 2 ID-GNB and AST-N255 cards (Bamford et al., 2010).

If indicated, repeat testing was done on bacterial organisms sub cultured onto agar plates, using Vitek 2, disc diffusion, or gradient diffusion test methods (bioMérieux,

Marcy l'Etoile, France). Fungal pathogens identified on Gram stain were grown onto Sabouraud dextrose agar. Identification and sensitivity testing of fungi were conducted using the Vitek 2 system with YST identification and AST-YS07 cards. Sensitivity results were analysed according to the Clinical and Laboratory Standards Institute criteria. *Coagulase negative staphylococcus*, *Bacillus species* and *micrococcus* species were considered "pathogens" if they grew in two or more collected blood culture bottles or if they grew in one bottle in a patient with clinical evidence of a bacteraemia; otherwise they were considered contaminants.

3.6 Data analysis and statistics

Descriptive statistics were used to summarise the baseline demographic and clinical characteristics of the study sample. Continuous variables (age) were summarised by mean, standard deviation, median, interquartile range, minimum and maximum values. Categorical variables (gender, type of malignancy) were summarised by frequency counts and percentages.

Organisms cultured were listed by frequency and percentages, the most prevalent organisms were identified with 95% confidence intervals and sensitivity patterns.
All statistical analysis was performed on SAS (SAS Institute Inc, Cary, NC, USA) release 9.4 or higher running under Microsoft windows. Statistical test (eg. Fisher exact tests for comparison of percentages) were two sided and p values of <0.05 were considered statistically significant.

The microbiology spectrum was stratified into multidrug-resistant positive and non-multidrug resistant organisms. Bacteria were considered multidrug-resistant if they were resistant (or with intermediate susceptibility) to at least three of the following antibiotic classes: piperacillin/tazobactam, cephalosporins, carbapenems, monobactams, aminoglycosides and/or fluoroquinolones for *Klebsiella pneumoniae* and if they were resistant to vancomycin for *Enterococcus faecium/faecalis* and resistant to methicillin for *Staphylococcus aureus* (MRSA)**.** The unknown sensitivity groups were contaminants (micrococcus, bacillus) which were negative on subsequent repeat cultures.

3.7 Significance of the study

This study quantified the bacterial spectrum and antibiotic susceptibility patterns locally, with the aim to improve clinical outcomes and mortality rates in chemotherapy-related febrile neutropenia.

3.8 Ethical considerations

This was a minimal risk study as this was a retrospective analysis of patient records. A waiver of individual parental consent or child assent was therefore obtained. Each patient record was assigned a unique study number. The list, linking the identifiable data, was kept separate in a secure space to protect patient privacy. Data was analysed anonymously, using only the unique study number during data analysis. The Turfloop Research Ethics Committee gave ethics approval, and the Pietersburg Hospital Clinical Director gave consent for the access to patient records.

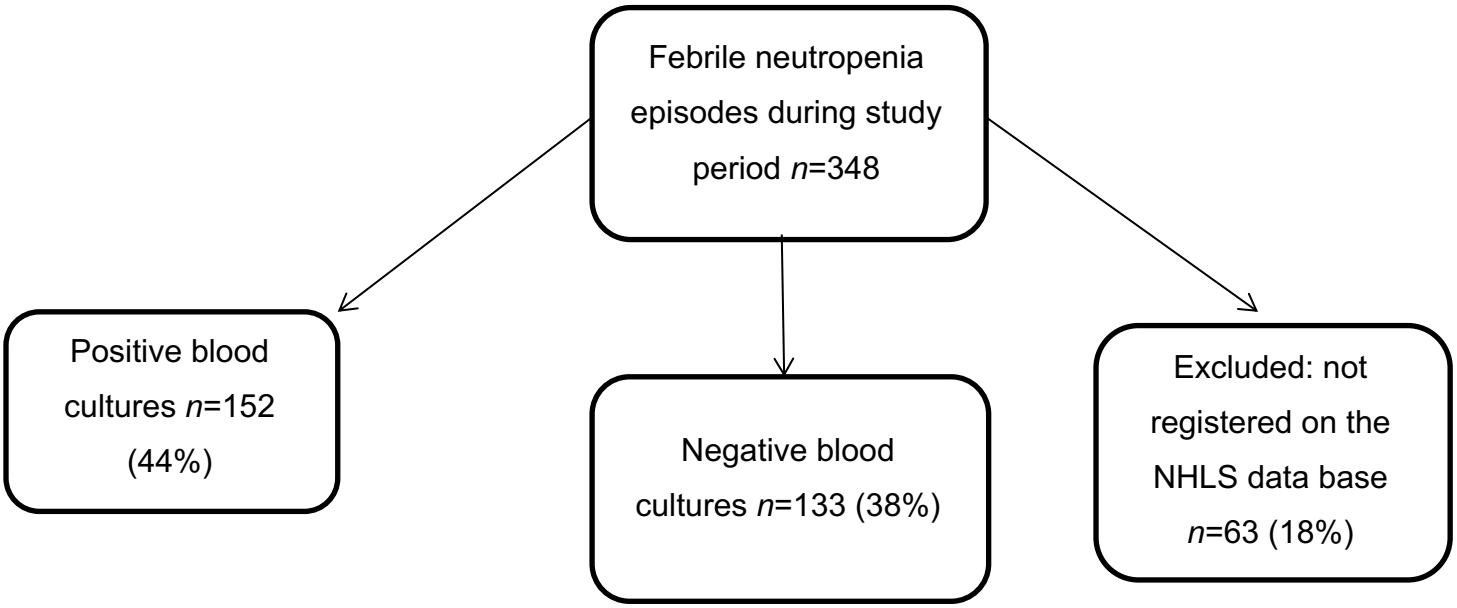
CHAPTER FOUR

4. PRESENTATION AND INTERPRETATION OF FINDINGS

4.1 Febrile neutropenia episodes

There were 348 episodes of febrile neutropenia during the study period. Of these 152 had positive blood cultures,133 were culture negative and in 63 instances no record of a culture was found (see figure 1).

Figure 1: Flow diagram of findings



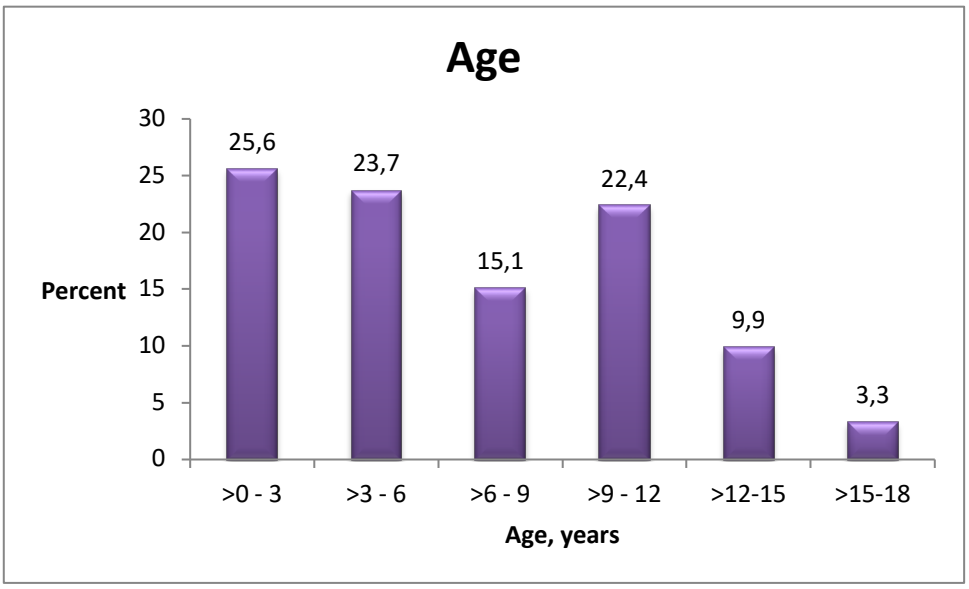
The majority were males (61.8%; *n*=93). Table 1 shows the detailed information on gender distribution.

Table 1: Gender

Gender	Frequency	Percentage
Male	93	61.8
Female	59	38.8
Total	152	100

The median age was 6years (IQ 3; 11, range 1 month to 17 years). The majority were between 0-years and 3-years of age (25, 6%) followed by 3-years to 6-years (23.7%), 9-years to 12-years (22.4%), 6-years to 9-years (15.1%), 12-years to 15-years (9.9%) (See figure 2)

Figure 2: Age distribution



4.2 Disease profile

Acute lymphoblastic leukemia was the most common childhood cancer diagnosis (33.6%), followed by Nephroblastoma (15.8%), Acute Myeloid leukemia (11.2%), NonHodgkin's Lymphoma (9.9%), Hodgkin's lymphoma (5.9%), Osteosarcoma (4.6%), Neuroblastoma (4.0%) and various other childhood cancers 15.3% (See figure 3 and figure 4)

Figure 3: Childhood cancer diagnoses

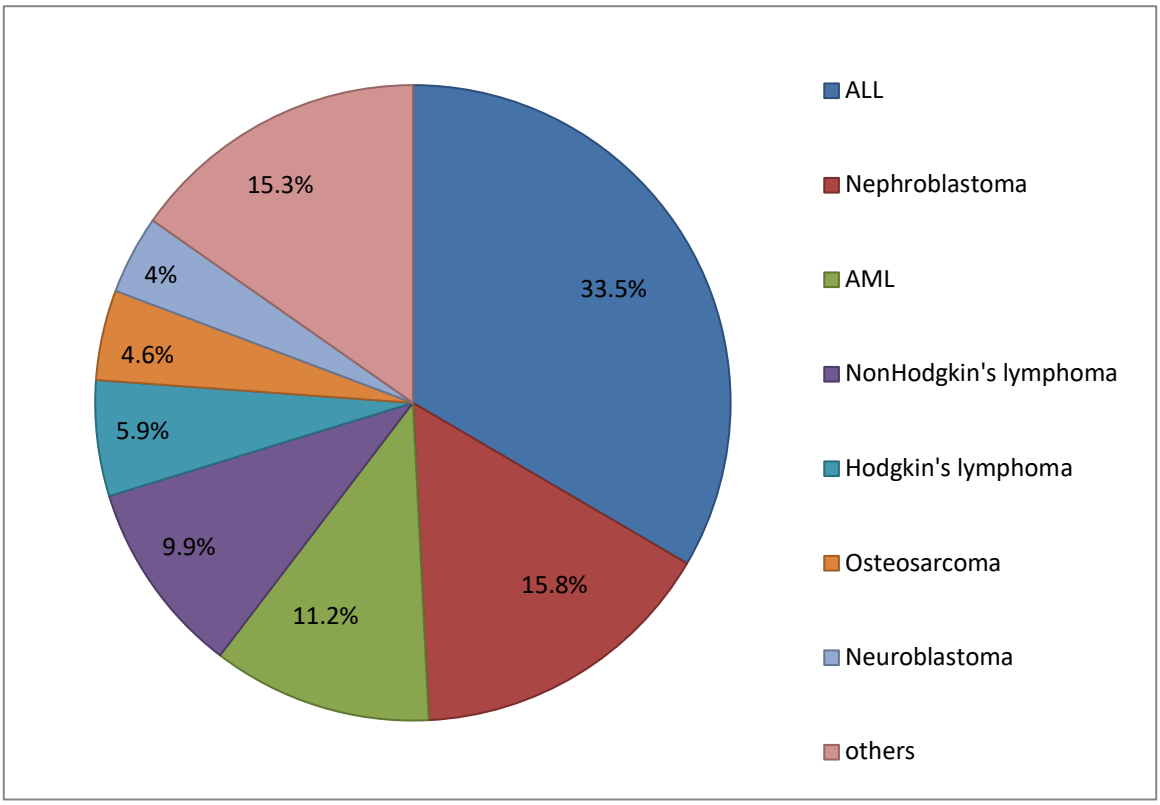
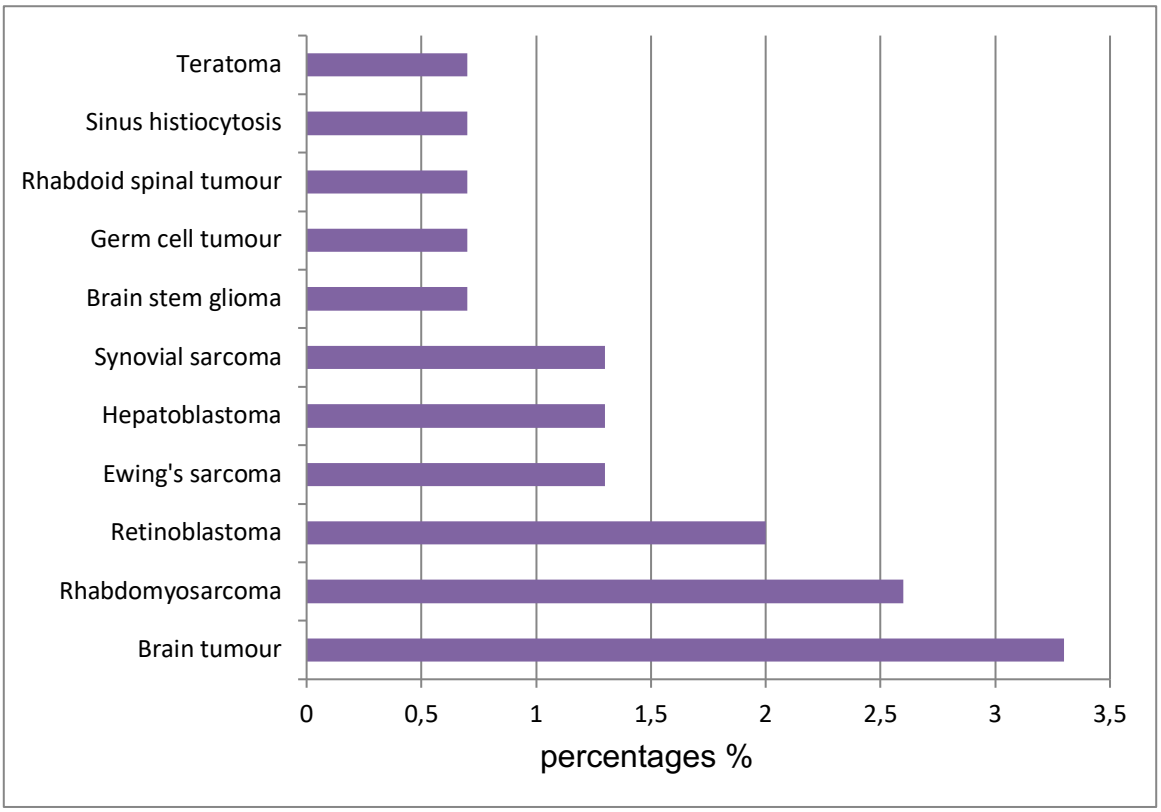


Figure 4: Other childhood cancers



4.3 Causative organisms of infection cultured

Gram-positive cocci were the predominant pathogens (45%) causing bacteraemia in neutropenic patients; especially *Coagulase negative staph* (21.6%). These *Coagulase negative staphylococci* episodes had at least two positive independent cultures during febrile neutropenia episodes.

Gram-negative bacteria, most often caused by *Klebsiella pneumoniae* (11.7%) was followed by fungal sepsis with *Candida albicans* and *Candida parapsilosis*, respectively (2.3%) each. Contaminants were identified (17.8%) in 27 instances. It became apparent that the number of isolates was more as there were two or more organisms cultured during an episode of febrile neutropenia. (See table 2)

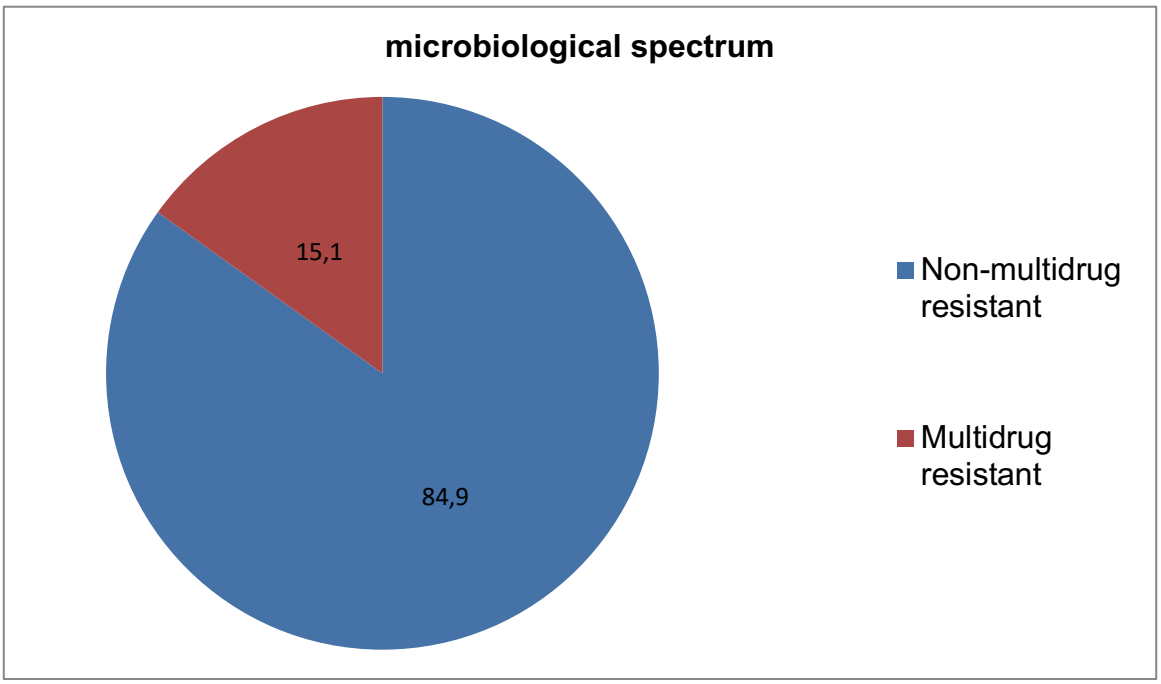
Table 2: Microbiological spectrum

Group	Organism	Frequency	Percentage
Gram positive	Coagulase negative staphylococcus	37	21.6
Gram positive	Staphylococcus aureus	8	5.3
Gram positive	Enterococcus faecium	6	4.1
Gram positive	Streptococcus viridans	5	3.5
Gram positive	Streptococcus pneumoniae	4	2.3
Gram positive	Streptococcus pyogenes	4	2.3
Gram positive	Streptococcus mitis	3	1.8
Gram positive	Enterococcus faecium VRE	3	1.9
Gram positive	Corynebacterium species	1	0.6
Gram positive	Enterococcus faecalis VRE	1	0.6
Gram positive	MRSA Staphylococcus	1	0.6
Gram positive	Micrococcus species	1	0.6
Gram negative	Klebsiella pneumoniae	20	11.7
Gram negative	Klebsiella pneumoniae CRE	16	10.7
Gram negative	Escherichia coli	5	2.9
Gram negative	Acinetobacter baumannii	3	1.8
Gram negative	Klebsiella oxytoca CRE	2	1.3
Gram negative	Pseudomonas aeruginosa	2	1.2
Gram negative	Enterobacter cloacae complex	2	1.2
Gram negative	Pantoea species	2	1.2
Gram negative	Sphingomonas paucimobilis	2	1.2
Gram negative	Acinetobacter Lwoffii	2	1.2
Gram negative	Enterobacter cloacae	1	0.6
Gram negative	Ochrobactrum anthropi	1	0.6
Gram negative	Salmonella enterica subsp enterica	1	0.6
Gram negative	Stenotrophomonas maltophilia	1	0.6

Fungal	Candida albicans	4	2.3
Fungal	Candida parapsilosis	4	2.3
Fungal	Candida famata	1	0.6
Fungal	Candida krusei	1	0.6
Fungal	Candida tropicalis	1	0.6
Contaminants	Bacillus species	17	9.9
Contaminants	Micrococcus species	10	7.9

84.9% organisms were Non-multidrug resistant and only 15.1% were multidrug resistant organisms. (See figure 5)

Figure 5: microbiological spectrum (%)

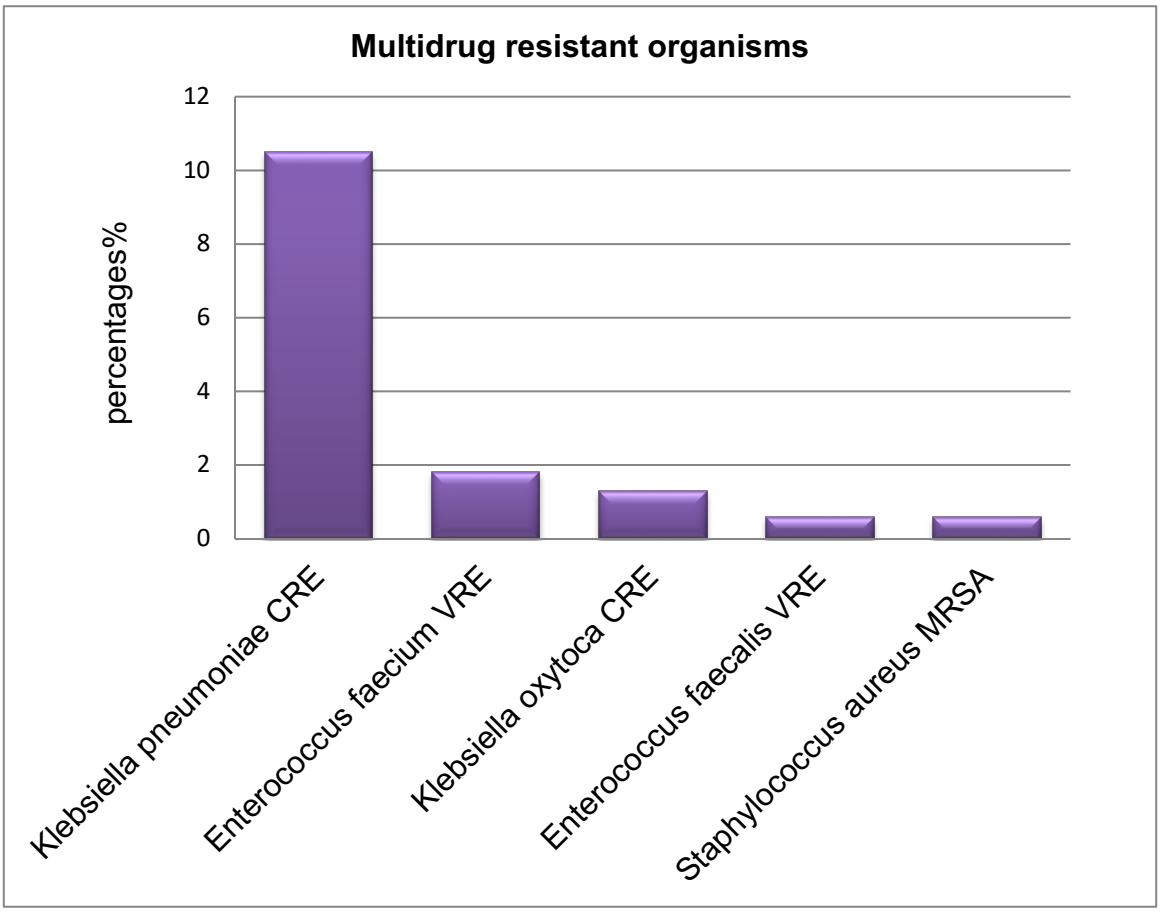


Carbapenem-resistant Enterobacteriaceae (CRE) *Klebsiella pneumoniae* was commonly cultured in 10.7% cases. Other multidrug resistant organisms included Vancomycin-resistant*Enterococcus faecium* (1.9%), Carbapenem-resistant *Klebsiella oxytoca* (1.3%), Vancomycin resistant *Enterococcus faecalis* (0.6%) and Methicillin- resistant *Staphylococcus aureus* (0.6%). (See table 3 and figure 6)

Table 3: Multidrug resistant organisms

Organism	Frequency	Percentage
Klebsiella pneumoniae CRE	16	10.7
Enterococcus faecium VRE	3	1.9
Klebsiella oxytoca CRE	2	1.3
Enterococcus faecalis VRE	1	0.6
Staphylococcus aureus MRSA	1	0.6

Figure 6: Multidrug resistant organisms



4.4 Antibiotic sensitivity profile

As part of the organism profile, we evaluated the bacterial sensitivity and resistance patterns (Table 4) to assess the efficacy of the current first line empiric antibiotic protocol in use namely piperacillin-tazobactam and amikacin. The predominant organisms were gram-positive bacteria. Based on the sensitivity testing all except 2 of 77 gram-positive organisms and 5 of 59 gram-negative organisms were resistant to piperacillin-tazobactam and 1 of 59 gram- negative organisms were resistant to Amikacin.

In conclusion this study found that the empirical antibiotic combination used in the POU was appropriate as the most common causative organisms were sensitive to both piperacillin/tazobactam and amikacin.

Table 4:

Antibiotic		Gram positive (n=77)							Gram negative (n=59)						
		N	Sensitive		Resistant		Intermediate		*N*	Sensitive		Resistant		Intermediate	
			n	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%
Beta-lactams	Penicillin	30	20	67	5	17	5	17	1	1	100				
	Ampicillin	50	40	80	8	16	2	4							
	Augmentin	1	1	100					8	2	25	4	50	2	25
	Cloxacillin	50	46	92	1	2	3	6							
Cephalosporins	Cefuroxime	2	1	50					10	4	40	4	40	2	20
	Ceftazidime	27	7	26	10	37	9	33	12	7	58	3	25	2	17
	Cefotaxime	9	7	77	1	11	1	11	7	4	57	3	43		
	Ceftriaxone	25	5	20	15	60	5	20	26	10	38	15	58	1	4
	Cefepime								14	10	71	3	21	1	7
Microlides	Erythromycin	65	55	85	10	15									
	Clindamycin	20	14	70	2	10	4	20							
	Tobramycin														
Carbapenems	Ertapenem								27	9	33	18	67		
	Meropenem	10	10	100					27	9	33	18	67		
	Imipenem								27	9	33	18	67		
Aminoglycoside	Gentamicin	4			3	75	1	25	9	3	33	6	67		
	Amikacin								55	40	73	1	2	14	25

Quinolone	Ciprofloxacillin	9	9	100					9	6	67	3	33		
others	Piperacillin-tazobactam	50	38	76	2	4	10	20	20	14	71	5	25	1	5
	Bactrim	10	7	70	2	20	1	10	13	1	8	10	77	2	15
	Vancomycin	50	38	76	4	8	8	16							
	Colistin								18	18	100				
	Linezolid	14	14	100											
	Rifampicin	5	3	60	2	40									

4.5 Outcomes of febrile neutropenic episodes:

During the study there were thirty deaths from bacteraemia, giving a case fatality rate of 8.6%. Twenty of these children, who died, had Carbapenem-resistant *Klebsiella pneumoniae* (66.7%); five had Vancomycin-resistant *Enterococcus faecium* (16.6%) and five had fungal bloodstream infection caused by *Candida parapsilosis* (16.6%). The majority, namely 318 (91.4%) were successfully treated for the febrile neutropenia episode with documented negative subsequent blood cultures after completion of antibiotic therapy.

CHAPTER FIVE

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study investigated causative organisms during febrile neutropenia episodes in children treated for cancer over a 5-year period in a single tertiary POU. Since the1970, a surge in the proportion of gram-positive bacteria has been reported as the cause of bacteraemia in paediatric oncology patients. (Van de Wetering, de Witte, Kremer, Offringa and Scholten, 2005); (Al-Mulla, Taj, El Shafie and Janahi, 2014). Like other studies the causative organisms of febrile neutropenia were predominantly gram-positive bacteria (45%) particularly *Coagulase negative staphylococcus* (21.6%) and *Staphylococcus aureus* (5.3%) in this study. (Selda, Elvan, Reyhan,Suleyan and Dilek, 2011); (Mvalo, Eley, Bamford, Stanley and Chagomerana, 2018); (Miedema, Winter, Ammann, Droz and Spanjaard, 2013); (Van de Wetering, Poole, Friedland and Caron, 2001). A European cohort reported 56.4% gram-positive causative bacteria, 18.9% gram-negative bacteria, fungi in 24.7% and no viral infections, in line with our findings (Selda et al, 2011).

A recent study conducted at Red Cross War Memorial Children's (RCWMCH) hospital in Western Cape, South Africa reported a predominance of gram-positive bacteria (49.1%) particularly *coagulase negative staphylococcus* (23.1%) and viridans group *streptococcus* (13.3%) (Mvalo et al, 2018). In contrast to this, a previous study conducted at the same institution found a predominance of gram-negative bacteria (58%), namely *Escherichia coli* (13.3%), *Klebsiella pneumoniae* (13.3%) and *Acinectobacter baumannii* (8.5%), demonstrating the potential that causative organisms might change from year to year or season to season (Lochan, Pillay, Bamford, Nuttall and Eley, 2017) .These findings were consistent with those reported by studies in East Africa which showed a predominance of gram-negative bacteria (66.7%) and a relatively low proportion of gram-positive bacteria of (33.3%). (Lubwama,Phipps,Najjuka,Kajumbula and Ddungu,2019); (Newman, Frimpong,

Donkor, Opintan and Asamoah-Adu, 2011). These significant differences in epidemiological profiles between the Low-to-middle-income countries and high-income countries may be related to differences in antibiotic stewardship, seasons or other unidentified factors (Lubwama et al, 2019).

While *E. coli* was the most common gram-negative organism described in numerous studies including Selda et al. (5.7%); Nihal et al. (39.5%); Mvalo et al. (11.0%), Baskaran et al. (21.9%) and Lochan et al. (13.3%) we documented 22.4% *Klebsiella pneumoniae* as the most commonly isolated gram-negative organism. Of concern, the contaminant prevalence of 17.8% in our study was more than the internationally acceptable contamination rate of 2% to 3% demonstrating the crucial need for refining aseptic blood culture taking measures and obtaining both central and peripheral blood cultures to differentiate infection from contamination and catheter related bloodstream infection as this differentiation determines antibiotic administration and catheter removal (Hall and Lyman, 2006).

Based on the sensitivity testing only 1.5% gram-positive organisms and 3.6% gram negative organisms were resistant to piperacillin-tazobactam, while 0.7% gram-negative organisms were resistant to amikacin. This high proportion of organisms sensitive to piperacillin-tazobactam and Amikacin was reassuring and therefore supported the use of the combination piperacillin-tazobactam and amikacin as the first line therapy in immunocompromised children with febrile neutropenia (Mvalo et al, 2018). Selda et al. reported that piperacillin-tazobactam showed highest in vitro activity against the common gram-negative isolates while amikacin showed highest sensitivity mainly to *Pseudomonas aeruginosa*. Similarly, RCWMCH also demonstrated 12% resistance to piperacillin-tazobactam which suggested that the combination therapy was an effective antibiotic choice in their POU (Mvalo et al, 2018). However, Lubwama et al., from Uganda East Africa reported 65% gram-negative resistance to piperacillin-tazobactam which proved that their current empirical therapy was not effective for the microbiological spectrum in their population. Lochan et al. in Cape Town, South Africa also failed to demonstrate susceptibility patterns to piperacillin-tazobactam in their cohort. A higher rate of antibiotic-resistant organisms were reported in East Africa with 85% multi-drug resistance rate among the Enterobacteriaceae including Carbapenem-resistant

Klebsiella pneumoniae (57.1%) and *E.coli* (36.4%) (Lubwama et al, 2019) and (Newman et al, 2011). However, in our study we reported a significantly lower multidrug resistance rate of 15.1%, with Carbapenem-resistant *Klebsiella pneumoniae* (10.7%) as the most common multidrug resistant organism. Relatively fewer resistance patterns of *Klebsiella pneumoniae* (20.9%) and *E coli* (30.2%) were reported in the European cohort (Selda et al., 2011).

In Uganda East Africa they reported 100% methicillin resistance to all staphylococcus species (Lubwama et al, 2019) while an Asian study in 2006 also demonstrated a high rate of methicillin resistance (76%) to its commonly isolated staphylococcus species (Baskaran et al., 2007) which was a significantly higher finding than that reported by Selda et al., (14.1%) in the European cohort. An interesting finding of our study is the lower prevalence of methicillin resistance (0.6%) among *Staphylococcus aureus* when compared with these studies.

We demonstrated a higher frequency of febrile neutropenia in patients with Acute lymphoblastic leukemia (33.5%) likely due to the more myelosuppressive chemotherapy which results in a longer duration of neutropenia which is a known risk factor for developing infection (Davis and Wilson, 2020). These findings are in keeping with those reported by Mvalo et al., Nihal et al., and Lubwama et al. While Baskaran et al., found that most patients suffered from acute myeloid leukemia (51.7%). Overall this demonstrated that patients with haematological malignancies particularly AML and ALL were shown to have higher bloodstream infection frequency than those with solid cancers.

In the present study, mortality as a result of blood stream infection occurred more frequently in multidrug resistant bacteria (83.4%) than fungal bloodstream infections (16.6%). The case fatality rate of 8.6% was similar to a study conducted at Chris Hani Baragwanath hospital in Johannesburg, South Africa investigating infections from1991-1995 which documented a case fatality rate of 8.5% (Van de Wetering et al., 2001). Whereas a recent study conducted at Red Cross War Memorial Children's Hospital, Western Cape in 2012 to 2014 recorded a low case fatality rate of 2% which was similar to that reported in Qatar over a study period of 2004 to 2011 which recorded a case fatality rate of 2.2%. (Mvalo et al., 2018; Al-Mulla et al., 2014)

In conclusion febrile neutropenia episodes were most often associated with gram-positive bacteria, sensitive to first line therapy with Piperacillin-tazobactam in combination with amikacin. The case fatality rate was 8.6% mainly due to multidrug resistant organisms.

A high index of suspicion with early addition of antifungal therapy in antibiotic regimen should be considered in children with febrile neutropenia who do not respond to first line therapy within 48 hours.

5.2 Limitations

The study was limited by its relatively small sample size and single-centre retrospective nature; there was missing data on patient records which made it difficult to trace microbiological data registered with the patient record number.
The lack of the mean inhibitory concentrations to identify the most effective antimicrobial agents and their effective concentrations especially for the multi-drug resistant bacterial isolates was a limitation in this study. There is however no reason to suspect that the missing data on patient records would have systematically biased the findings in an adverse fashion.

5.3 Recommendations

Febrile neutropenia is the most significant factor contributing to the mortality rate of children diagnosed with cancer. Early risk stratification, blood culture collection and initiation of broad-spectrum antibiotics should be the treatment goal to prevent morbidity and mortality associated with delayed treatment administration. Based on our study evidence we have proven that our first line empirical treatment is effective against our local bacterial profile.

Due to the shift from gram negative to gram positive predominance, we recommend obtaining both central and peripheral blood cultures to differentiate infection from contamination and primary bacteraemia from catheter related bloodstream infection as these distinctions will affect treatment decisions, including antibiotic administration and catheter removal.

Further surveillance and local studies are recommended to monitor our antimicrobial susceptibility and resistance patterns especially multidrug resistant organisms that have emerged in our study as this poses a significant outcome risk in our setting. We hope that this study will ignite a new interest in the research of local microbial spectrum and antibiotic profiling in pediatric cancer patients with febrile neutropenia in a low socioeconomic setting.

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Annexure 1: Data collection sheet

PART1: PATIENT RELATED PARAMETERS

Study number	
Date of admission	
Age	
gender	

PART2: DISEASE RELATED PARAMETERS

Type of malignancy	
Stage of malignancy	
Date of last chemotherapy administration	

PART3: LABORATORY PARAMETERS

Blood culture results	positive	negative	contaminant
organisms			
Antibiotic sensitivity			

Annexure 2: TREC Ethics clearance certificate

University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 3935, Fax: (015) 268 2306, Email:anastasia.ngobe@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING: 11 May 2021

PROJECT NUMBER: TREC/75/2021: PG

PROJECT:

Title:	Causative Infections in Childhood Cancer Patients with Febrile neutropenia in Pietersburg Hospital, Limpopo Province
Researcher:	NE Nonkonyana
Supervisor:	Dr V Netshituni
Co-Supervisor/s:	Prof M Kruger
School:	Medicine
Degree:	Master of Medicine in Paediatrics and Child Health

PROF P MASOKO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE
The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:

i) **This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.**

ii) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.**

iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

Finding solutions for Africa

Annexure 3: Permission letter from Limpopo Department of Health

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Department of Health

Ref	:	LP_2021-05-011
Enquires	:	Ms PF Mahlokwane Tel : 015-293 6028
Email	:	Phoebe.Mahlokwane@dhsd.limpopo.gov.za

Nomsa Nonkonyana Mothiba
PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;
Causative infections in childhood cancer patients with febrile neutropenia in Pietersburg hospital, Limpopo province

1. Permission to conduct research study as per your research proposal is hereby Granted.
2. Kindly note the following:

a) Present this letter of permission to the institution supervisor/s a week before the study is conducted.
b) In the course of your study, there should be no action that disrupts the routine services, or incur any cost on the Department.
c) After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
d) The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
e) The approval is only valid for a 1-year period.
f) If the proposal has been amended, a new approval should be sought from the Department of Health
g) Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated

08/06/2021

Private Bag X9302 Polokwane
Fidel Castro Ruz House, 18 College Street. Polokwane 0700. Tel: 015 293 6000/12. Fax: 015 293 6211.
Website: http/www.limpopo.gov.za

Annexure 4: Letter from editor

LEBOMA INVESTMENTS (PTY) LTD

REGISTRATION NUMBER: 2018 / 299676 / 07

TO WHOM IT MAY CONCERN

This letter serves to confirm that I, **Prof T.W Molotja**, have proofread and edited the research report for **NOMSA EDITH MOTHIBA,** student number **200902622** entitled:

CAUSATIVE INFECTIONS IN CHILDHOOD CANCER PATIENTS WITH FEBRILE NEUTROPENIA IN PIETERSBURG HOSPITAL, LIMPOPO PROVINCE, SOUTH AFRICA

The report is edited focusing on the following**:**

- Coherent writing.
- Eliminating spelling errors.
- Fluency in reading.
- Academic writing.

I therefore recommend for its submission.

Yours Sincerely Date: 24/09/2021