BLOOD STREAM INFECTIONS IN ONCOLOGY PATIENTS AT RED CROSS WAR MEMORIAL CHILDREN`S HOSPITAL, CAPE TOWN

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Declaration

I , Tisungane Knox Titus Mvalo, hereby declare that the work on this dissertation/thesis is based on my original work (except where otherwise indicated by acknowledgements) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to this or any other university. No plagiarism has occurred in the work reported in this dissertation.

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Abstract

Background: Infections cause significant morbidity and mortality in children with cancer, which may be related to the cancer or treatment received. There is paucity of data on the epidemiology of bloodstream infection (BSI) in sub-Saharan Africa. To address this knowledge gap, the present study was conducted at Red Cross War Memorial Children`s Hospital (RCWMCH) in Cape Town, South Africa.

Methods:

Structured literature review: From 1 April 2016 to 31 May 2016 a PubMed search was undertaken on BSI in Paediatric Oncology. The search string used was (bacteraemia OR blood stream infection) AND (paediatric OR pediatric) AND (oncology). Studies that did not describe infection patterns, risk factors for infection, morbidity/mortality, articles not in English and those exclusively describing neonatal or ICU patients were excluded from full review.

Retrospective cohort study: A retrospective cohort study was conducted at the haematology-oncology unit of RCWMCH. All positive blood cultures from RCWMCH oncology patients taken between 1 January 2012 and 31 December 2014 were retrieved to identify patients who had BSI.

Results:

Structured literature review: 508 abstracts / articles were initially retrieved and screened. 478 studies were excluded as per the literature review exclusion criteria. Thus, 30 articles were included in full analysis, 17 retrospective studies, 4 prospective multicentre studies, 6 prospective single centre studies, 2 systematic reviews and 1 case report. All were observational studies. This literature review showed that BSI is a frequent and important cause of morbidity and mortality in paediatric oncology. Gram-positive bacteria was noted to be the leading type of pathogen causing BSI. Increased risk of BSI may be from the cancer itself, chemotherapy, hospitalisation, central venous catheter insertion, and oncology patients were at risk of multi-drug resistant infection. Research gaps noted included paucity of studies from Sub-Saharan Africa, limited analysis of the antimicrobial susceptibility of causative microorganisms and limited description of fungal BSI in oncology patients.

Retrospective cohort study: From 436 positive blood culture results, 150 BSI episodes were identified amongst 89 patients; 49.1% of the culture isolates were Gram-positive bacteria, 41.6 were Gram-negative bacteria and 9.3% were fungal. Coagulase Negative *Staphylococcus* and *Viridans Group Streptococcus* were the most common Gram-positive isolates, and *Escherichia coli* and *Klebsiella species* the commonest Gram-negative isolates. The majority of BSI episodes occurred in patients with haematological malignancies (74%), in the presence of severe neutropaenia (76.4%) and whilst on or following chemotherapy (88%). Complications occurred in 14% of the BSI episodes. Fungal infections had the highest prevalence of complications (21.4%). Three children died during BSI as a result of multidrug resistant isolates, giving a case-fatality rate of 2%.

Conclusion: The findings of our cohort study show that BSI are mainly caused by Gram-positive bacteria and associated with a low case-fatality rate. The results of this study are consistent with worldwide experience of BSI in paediatric oncology patients. This study provides an understanding of the spectrum of organisms causing BSI and the outcome of BSI in a sub-Saharan African context.

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List of abbreviations:

ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ANC	Absolute neutrophil count
BSI	Blood stream infection
CABSI	Catheter associated blood stream infection
CFR	Case fatality rate
CLABSI	Central line associated blood stream infection
CONS	Coagulase negative staphylococcus
CVC	Central venous catheter
CVP	Central venous pressure
ELISA	Enzyme linked immunosorbent assay
HAI	Hospital-acquired infection
HIV	Human immunodeficiency virus
HSCT	Haematopoietic stem cell transplant
ICU	Intensive care unit
NHL	Non-Hodgkin lymphoma
PCR	Polymerase chain reaction
IR	Infection Risk
NHLS	National Health Laboratory Services
RCWMCH	Red Cross War Memorial Children`s Hospital
TED	Total external device
TID	Totally implantable device
VAD	Venous access device
VGS	Viridans group streptococcus

Chapter 1: Introduction and literature review

1.1 Introduction

Infections are a major contributing factor to morbidity and mortality in children with cancer.[1-3] This may be due to risk factors from the cancer itself or factors related to treatment. These main groups of factors may increase risk of infection by decreasing the effectiveness of innate immunity, decreasing the functional capacity of the acquired immune system and increased exposure to pathogens.[4]

The innate system may be weakened by decreased integrity of epithelial barriers, decreased ability to clear secretions or decreased function of leukocytes.[1] Chemotherapy, radiotherapy, prophylactic antibiotics, surgery, hematopoietic stem cell transplant (HSCT) and the cancer itself may all contribute to this.[1,2,5,6] Neutropaenia in cancer patients is associated with increased risk of infections and poor outcomes of the infections. These risks are more pronounced with prolonged neutropaenia and severe neutropaenia.[7] Severe and prolonged neutropaenia is also associated with poor infectious episode outcomes.[8] In cancer patients the adaptive immune system may be suppressed due to decreased levels B lymphocytes and T lymphocytes that would normally produce an immunological memory based antigen detection response, resulting in reduced humoral and cell mediated immunity. Reduced functional capacity of the adaptive immune system leads to reduced capacity to produce antibodies, reduced activation of the complement system and reduced activation of natural killer lymphocytes and macrophages. Bone marrow suppression or infiltration by the cancer itself may cause these immune alterations. Chemotherapy, radiotherapy and HSCT may also cause dysfunction of the adaptive immune response.[9,10]

Haematological cancers have been shown to confer a greater infection risk than solid tumours.[11] This is more prominent in patients who are neutropaenic as a result of bone marrow infiltration, craniospinal radiotherapy or increasingly intensive chemotherapy regimens.[12,13]

Cancer patients usually have prolonged hospital stay, indwelling vascular catheters for chemotherapy, multiple hospital procedures and neutropaenia which put them at risk of both community- and hospital-acquired infection.[4,6,8,12,14-18] Hospital-acquired infection (HAI) is associated with higher morbidity and mortality, and frequently caused by multidrug resistant pathogens.[4,11,12]

These same factors that increase their risk for developing infections may also increase the risk of complicated infections. Cancer patients may have different infection patterns caused by different pathogens in comparison to the general paediatric population. For instance, patients with mucositis have an increased risk of developing Viridans group streptococcal (VGS) infections particularly in acute myeloid leukaemia (AML).[19-21] *Coagulase negative Staphylococci* (CONS) and *Bacillus cereus* have also been shown to occur with increased frequency in oncology patients.[8,21] In the general paediatric population these oral isolates are not a common cause of systemic infection.[22] The presence of specific types of central venous catheters (CVCs) may also contribute to different infection patterns.[22,24] The risk of catheter-related infection itself varies with the type of CVC.[11,15]CVC related infections may also increase the risk of poor outcome since nosocomial infections that may develop from colonised CVC are frequently caused by multi-drug resistant pathogens.[16] Whereas oral

prophylactic antibiotics reduce infection incidence they may also alter the pattern of infection due to alteration of gastroenterological tract colonisation.[25]

1.2 Purpose of Literature review:

1- To describe the spectrum of bacteria and fungi causing bloodstream infections (BSI) amongst paediatric oncology patients.

2- To evaluate the extent of morbidity and mortality caused by BSI in paediatric oncology patients.

3- To assess risk factors for BSI in paediatric oncology patients.

4- To evaluate the antibiotic susceptibility patterns of blood culture isolates in paediatric oncology patients.

5- To identify gaps in the knowledge of BSI in paediatric oncology patients within an African setting.

1.3 Search strategy.

An internet search strategy was undertaken on PubMed. The search string used was (bacteraemia OR blood stream infection) AND (paediatric OR pediatric) AND (oncology). This search came out with 508 articles. This literature search was completed between 1 April 2016 and 31 May 2016.

Abstracts were reviewed for inclusion for full literature review.

Criteria for inclusion included

1- Articles in English
2- Studies needed to be done in a paediatric cancer population. A population was considered to be paediatric if the patients were of the aged 18 years or less. If the population was mixed, a separate description of the paediatric population was needed.
3- Studies needed to have described at least one of the following; infection patterns, risk factors for infection, antibiotic susceptibility patterns, morbidity or mortality patterns.

Studies done exclusively in a neonatal or paediatric intensive care unit were excluded to reduce bias as they may have a different infection pattern.

Figure 1: Selection of eligible articles for review

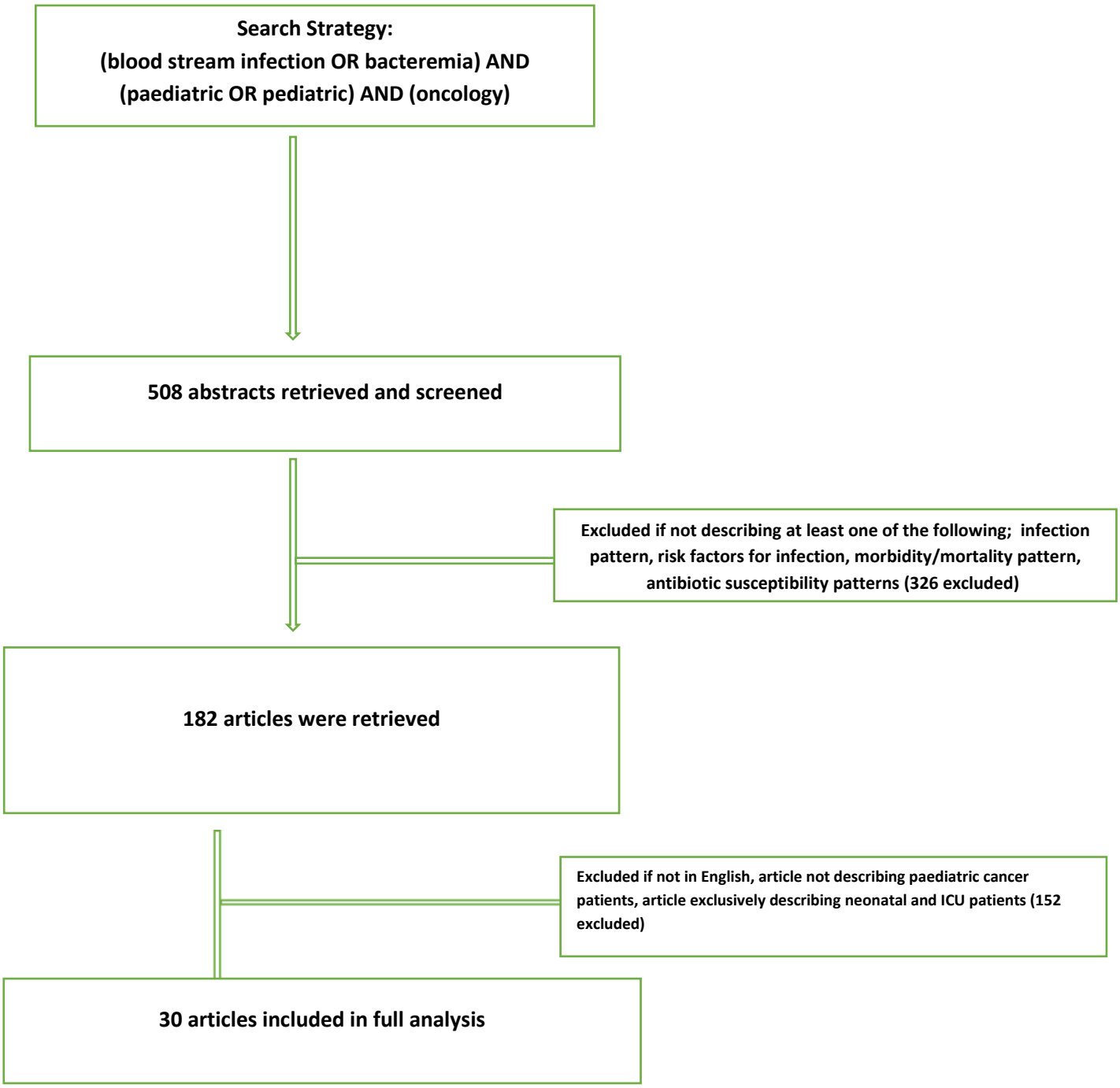

Table 1: Summary of reviewed literature

First Author	Study location and date	Study design	Study window	Outcome investigated	Study population	Number of patients	Number of infections	Comment
Adler A (23)	Tel Aviv, Israel. 2005	Prospective case cohort analysis	2000-2003	To investigate the infectious complications associated with Hickman and implantable ports in children	Paediatric Haematology and Oncology patients with CVCs	281	207	-Tunnelled External CVCs had increased risk of CABSI compared to Total Implantable CVCs -Haematopoietic Stem Cell Transplant a risk factor for CABSI development
Alexander S (2)	Toronto, Canada 2011	Systematic review	2011	Prevention of Bacterial infection in Paediatric Oncology	Paediatric oncology patients	-	-	-Central lines having prophylactic antibiotics administered had significant effect in preventing BSI -25% of patients colonised with bacteria may eventually develop a BSI.
Allen RC (16)	Mexico, 2007	Prospective case analysis	2004	Investigation of risk determinants for Catheter Associated (CABSI`s) in children and young adults with cancer	Paediatric and young adult oncology patients with CVCs	139	58	-Tunnel external CVCs having increased risk of CABSI than Total Implantable CVCs -Hospitalisation was shown to be a risk factor for CABSI.
Al-Mulla NA(11)	Qatar. 2014	Retrospective case analysis	2004-2011	Burden of infection and antibiotic susceptibility in paediatric oncology	Paediatric oncology inpatients	70	111	-Most infection in non-neutropaenic patients. -Multi drug resistant infection leading to mortality
Al-Tonbray YA (12)	Mansoura, Egypt. 2011.	Retrospective case analysis	2007-2008 (January to January)	Nosocomial Infections pattern and susceptibility patterns	Paediatric oncology inpatients	1564	234	High antibiotic resistance rates for nosocomial infections
Ammann RA (39)	Germany and Switzerland, 2014	Prospective multicentre surveillance study	November 2007 to June 2010	-To identify risk factors for BSI in Paediatric cancer patients	-Paediatric cancer patients	770 patients	179 BSI amongst 142 patients	-ALL, AML, Broviac lines and relapse malignancy patients have increased risk of developing BSI. Relapse malignancy shown to be an independent risk factor for BSI
Bhattacharyya A (31)	India, 2014	Retrospective study	January-December 2013	-The epidemiology of infections in patients with cancer	-Paediatric patients with haematological malignancies, solid tumours and post stem cell transplant.	262 patients	110 BSI	-Gram-negative infections were the predominant cause of BSI in this population. -Multidrug resistant Gram-negative bacteria levels were high and this attributed to high morbidity and mortality levels.

Castagnola E (19)	Italy. 2005	Retrospective case review and analysis	1988-2000	To investigate incidence rates of BSI and Invasive mycoses in Acute leukaemia patients on chemotherapy	Paediatric acute leukaemia patients (352)	127	175	-AML patients had highest infection incidence in comparison to other leukaemia. -Correlation between infection risk and chemotherapy intensity identified.
Christenson JC (21)	Utah USA, 1999	Case Reports	April and May 1998	-Epidemiologic findings and course of *Bacillus cereus* infections amongst paediatric oncology patients	-Paediatric oncology patients with *Bacillus cereus* infections.	3 patients	3	-Sporadic infections from Bacillus cereus immunosuppression do occur and can be associated with significant morbidity.
Delebarre M (7)	Lille France, 2014	Retrospective cohort study	2005-2006	-Identifying variables useful for predicting severe infection in febrile neutropaenia	-Paediatric oncology patients with chemotherapy induced febrile neutropenia	160 patients	60 cases of severe infections identified from 372 episodes of febrile neutropenia.	-Prolonged neutropaenia, high-grade fever in neutropaenia, haematological cancer and high levels of C Reactive Protein associated with occurrence of severe infection.
Esbenshade AJ (28)	Tennessee, USA 2014	Retrospective cohort study	2007-2010	-To identify and develop a prediction model for diagnosing BSI in febrile non neutropaenic patients with cancer	-Paediatric oncology patients	463 patients	-932 episodes of fever and 91 cases of BSI	- In absence of neutropaenia patients with external VAD, young age and those presenting with febrile illness are at increased risk of having BSI
Escande MC (26)	France. 1998	Prospective multi centre case analysis	November 1994	Epidemiology of bacteraemia in cancer patients	Adult and Paediatric cancer patients from 70 centres amongst 54 hospitals	403	494	-64% cases were nosocomial. -Aetiology of BSI was not shown to be related to cancer type.
El-Mahallaway H (1)	Cairo, Egypt. 2003.	Retrospective case review	1999	Determinants of Blood stream infections in Paediatric cancer	Paediatric oncology inpatients	250	328	High resistance rates to Gram-negative isolates
El-Mahallawy HA, (32)	Egypt, 2016	Retrospective cohort study	June-December 2012	-The risk factors and outcome of ESKAPE pathogen BSI in comparison to CONS BSI.	-Paediatric oncology patients with positive blood cultures from ESKAPE pathogens or CONS.	72 patients with ESKAPE positive blood cultures 166 patients with	81 episodes of ESKAPE pathogen BSI 135 episodes of CONS BSI	-40% of BSI were caused by CONS whilst ESKAPE pathogens attributed to 24% of the BSI. -ESKAPE pathogens were the cause of 75% all hospital acquired infections. -ESKAPE pathogens were shown to have significantly higher morbidity and mortality than CONS infections.

						CONS positive blood cultures	336 episodes of BSI from all causes	
Huang WT (22)	Taiwan,2007	Retrospective chart review	1998-2004	To describe clinical features and complications of viridans streptococci BSI in Paediatric Haemato-Oncology	Paediatric Haemato-Oncology patients	25	26	-Viridans streptococci BSI significantly higher in AML patients -Viridans streptococci BSI with high morbidity, antibiotic resistance and mortality in haemato-Oncology patients.
Johannsen KH (20)	Denmark. 2012	Retrospective case analysis	2004-2011	To evaluate infections in children with AML per the (NOPHO)-AML-2004 protocol	AML patients	43	99	-VGS most common isolate in AML but poor susceptibility to Penicillin -VGS infection not related to cytarabine dose.
Laws HJ (3)	Dusseldorf, Germany 2000	Retrospective case review	2006	To investigate and describe trends of infections amongst children with cancer in 2000 in comparison to 1980/1981	Paediatric cancer patients	157 patients in 2000 versus 125 patients in 1980/1981	249 in 2000 versus 133 in 1980/1981	-Gram-positive infections were predominant in both timeframes. -Similar infection mortality rates were observed between these time frames.
Neilsen MJ (38)	Liverpool UK, 2015	Retrospective study	2003-2013	-Characteristics, outcome and resistance patterns of VGS bacteraemia in children receiving treatment for malignancy or HSCT	-Paediatric haematology oncology patients with VGS bacteraemia	-46 patients	-54 VGS BSI	-30% of cases were resistant to Penicillin. -Mortality rate of 5.6% was noted due to VGS BSI -VGS BSI are more potentially fatal in paediatric haematology patients.
Newman N (24)	Beersheba, Israel. 2012	Retrospective case review and analysis	1998-2008	To investigate epidemiological and microbiological aspects of CVC associated BSI	All paediatric cancer patients with CVC in study window	93	178	-Higher infection rates with Hickman lines in comparison to Port-A-Cath lines. -Different pathogen distribution with different CVC types.
Orozco HH (4)	Mexico. 2013	Prospective surveillance	2004-2009	To investigate the incidence of HAI and patients at greatest risk for HAI	Paediatric cancer patients	9420	-409 HAI -272 BSI	-Risk factors reported for HAI were prolonged hospitalisation, haematological malignancy, neutropenia and malignancy relapse -Gram-negative bacteria aetiological factor for majority of HAI identified.

Rinke ML (5)	New York, USA, 2013	Prospective case analysis	2009-2010	To compare the burden of CLABSI between ambulatory and inpatient paediatric Oncology patients	Paediatric Oncology patients with CVCs	319	74	-Outpatients had CLABSI more commonly than inpatients with incidence rate ratio of 3 -Tunnelled external CVCs had increased risk of CLABSI than patients with Total implantable devices with an incidence rate ratio of 20.6
Simon A (18)	Germany and Switzerland	Prospective multicentre surveillance for HAI and Fever of Unknown Origin.	2001-2005	-To investigate and describe hospital acquired infections in paediatric cancer patients.	-Paediatric cancer inpatients from 7 oncology centres	411 patients	727 hospital acquired infections and nosocomial fever of unknown origin cases.	-CVC and neutropenia associated with increased risk of hospital acquired infections. -Paediatric cancer patients as a population have increased risk of hospital-acquired infections.
Tsai HC (29)	Taipei, Taiwan. 2014	Retrospective case analysis	2009-2013	To analyse prevalence of CLABSI and the effectiveness of ALT in paediatrics	Paediatric haematology- oncology patients with CVC`s.	157 BSI	497	-AML patients had highest infection density. -Candidaemia had highest mortality.
Urrea M (16)	Barcelona, Spain 2004	Prospective surveillance study	March – May 2001	-To investigate and describe the incidence of nosocomial infections in children with neoplastic disease	-Paediatric cancer patients hospitalised to the haematology-oncology unit	51 inpatients	18 nosocomial infections amongst 12 patients.	-ALL patients had highest nosocomial infection rate. -CONS was the commonest individual cause of nosocomial infections. -CVC insertion and parenteral nutrition associated with increased risk of nosocomial infection.
Van de Wetering MD (15)	Johannesburg, South Africa, 2001	Retrospective case review	1991-1995	Morbidity and mortality of infections in Paediatric oncology	Paediatric oncology inpatients	83	200	Mortality rate 8.5%, predominantly Gram-negative cause.
Van de Wetering MD (25)	Netherlands, 2004	Systematic review of randomised controlled trials.	1966-2002	-To assess the effectiveness of oral prophylactic antibiotics to decrease bacteraemia and infection related mortality in neutropaenic oncology patients.	-Paediatric oncology patients	Patients from 22 clinical trials	-	-Incidence of Gram-negative infections significantly reduced with oral quinolone antibiotic prophylaxis. -Oral prophylactic antibiotics shown to decrease infection related mortality.
Viscoli C (6)	Italy. 1999	Prospective multi centre surveillance	1995 (January to December)	Main clinical features and outcomes of BSI in children with cancer	Paediatric cancer patients from 18 centres	156	191	-High Gram-negative infections with Hickman lines -High mortality associated polymicrobial infections, fungaemia and HSCT.

Wehl G (8)	Austria, 1997	Retrospective case analysis	1986-1995	Investigation of the types, severity, frequency of febrile infectious complications in Paediatric cancer patients	Paediatric oncology patients receiving chemotherapy	357	217	-Increase in Gram-positive infections with time in Paediatric oncology patients on chemotherapy, particularly CONS. -Gram-negative infectious generally with high resistance pattern to antibiotics. -Patients with prolonged neutropaenia had poor outcomes of infections.
Wolf J (14)	Tennessee, USA 2014	Retrospective cohort study	2006-2012	-To investigate if there is any benefit of antibiotic lock therapy with central line related blood stream infection	Paediatric cancer patients with central lines receiving systemic antibiotic lock therapy with catheter related BSI	-	38 CRBSI patients treated with adjunctive lock therapy versus 73 episodes of CRBSI treated with standard therapy	-CRBSI are common sequelae of CVC insertion and ay occur in 1 in 4 CVCs inserted. No benefit of adjunctive antibiotic lock therapy on top of the standard therapy alone.
Yacobovich J (13)	Israel 2014	Prospective cohort analysis	2006-2008	To assess the host and CVC related risk factors for Blood stream infections in Paediatric oncology	Paediatric oncology on chemotherapy or undergoing Bone marrow transplant	262	152	-Type of CVC and type of cancer shown to have risks for specific type of microbiologic cause. -HSCT and younger age increase risk for BSI.

Abbreviations: ALL, acute lymphoblastic leukaemia; ALT, antimicrobial lock therapy; AML, acute myeloid leukaemia; BSI, bloodstream infection; CABSI, catheter associated bloodstream infection; CLABSI, central line associated bloodstream infection; CONS, coagulase negative staphylococcus; CRBSI, catheter related bloodstream infection; CVC, central venous catheter; ESKAPE pathogens, e*nterobacter spp, methicillin resistant staphylococcus aureus, klebsiella pneumonia, acinetobacter baumannii, pseudomonas aureginosa, enterococcus spectrum* of pathogens; HAI, hospital acquired infections; HSCT, haematopoietic stem cell transplant; NOPHO, Nordic society of paediatric haematology and oncology; VAD, venous access device; VGS, viridans group streptococcus.

Quality of Evidence

Thirty articles were critically reviewed. Of these 17 were retrospective studies, 4 were prospective multicentre studies, 6 were prospective single centre case series studies, 2 were systematic reviews and 1 case report.

All these articles described observational studies. From these thirty studies, 20 reported on BSI in a whole paediatric oncology cohort, 6 focussed on CVC related or associated BSI and 4 described BSI in haematological cancer cohorts. The majority of the studies were observational retrospective cohort studies, possibly due to the relative ease of conducting such studies. Although observational studies are low quality studies, these studies originated from a variety of geographical settings and populations.

There were consistencies in the findings in most of the articles as reported below including the pattern of common causes of BSI in paediatric oncology patients and the risk factors for BSI such as haematological cancers. These consistent findings across studies suggest that the literature is reliable.

1.4 Interpretation of literature

1- Pattern of pathogens causing BSI in paediatric oncology patients.
2- Characteristics of cancer patients in relation to the BSI.
3- BSI-associated morbidity and mortality.
4- Variation of BSI pathogens in relation to cancer diagnosis.
5- Factors influencing morbidity and mortality in cancer patients with BSI.
6- BSI-associated risk factors.
7- Antibiotic susceptibility patterns.

1.4.1 Pattern of pathogens causing BSI in paediatric oncology patients

Most BSI in children with cancer has been shown to be caused by Gram-positive pathogens.[8] This has been attributed to the intensive chemotherapeutic regimens, antibiotic prophylaxis that results in changes to the bacterial flora of the gastrointestinal tract, mucositis that may be related to chemotherapy or oral infections in neutropaenic patients, and indwelling CVCs.[20,26]

For the period 2008-2012, 56.6 % of all pathogens isolated from blood culture specimens at RCWMCH were Gram-negative bacteria. This analysis was not confined to oncology patients but included results from all children admitted to RCWMCH.[27] In contrast, Gram-positive bacteria are the predominant group of pathogens that cause BSI in paediatric oncology patients.[20] The most common Gram-positive bacteria being coagulase negative *Staphylococcus* (CONS) and VGS.[11]

A study in paediatric haematology-oncology patients completed in Johannesburg between 1991 and 1995 showed that Gram-positive bacteria were the main cause of BSI accounting for 70% of positive blood cultures isolates. Whereas Gram-negative bacteria (20%) and fungi (10%) were responsible for the remaining positive blood cultures.[15] This study only analysed patients with positive blood cultures and therefore did not describe the aetiology all invasive infection episodes.

Risk factors have been described for Gram-negative BSI in paediatric oncology patients. Studies in Israel showed that Gram-negative infections are related to the presence of tunnel external CVC devices.[23,24] Furthermore, a Mexican study showed predominance of Gram-negative infections in HAI.[4]

Fungal aetiology of BSI was not documented in all articles reviewed. Although not a major cause of BSI, fungal BSI is challenging to treat particularly in patients with haematological malignancies.[6,19]

1.4.2 Characteristics of cancer patients in relation to the BSI

Oncology patients are known to be at increased risk of developing infection when compared to patients with non-malignant conditions .[4,12,20,28] Al-Mulla et al demonstrated that haematological malignancy patients have a higher risk of developing BSI than patients with other malignancies.[11] In this study in Qatar 54% of BSI occurred in patients with leukaemia, 47% in children with acute lymphoblastic leukaemia (ALL) and 7% in those with AML. The difference in the distribution of BSI between patients with leukaemia and those with lymphomas or solid tumours was statistically significant. This could be attributed to the primary oncological malignancy or aggressive chemotherapy such patients undergo. This difference in occurrence of BSI between types of malignancies may be reduced if aggressive chemotherapy regimens are also administered to patients with solid tumours as shown by Escande et al in France.[26] When patients with solid tumours received aggressive chemotherapy the difference in the distribution of BSI in relation to the underlying diagnosis was not shown to be statistically significant.

Separate studies have also shown that patients with AML have increased risk of infection in comparison to other malignancies. Tsai et al reported that patients with AML in Taiwan experience the highest infection density (5.26 BSI 1000 patient days).[29] Similarly, Castagnola et al in Italy demonstrated that patients with AML have a higher infection risk (IR) compared to those with ALL.[19] In this study the overall IR, reported as the number of BSI episodes per 100 days, was 0.106. Patients with AML and ALL had infection risks of 0.315 and 0.092 respectively. The IR also differed in relation to the phase of chemotherapy, with a highest IR occurring in patients receiving second line intensive chemotherapy. This demonstrates that not only may the primary oncological diagnosis have an impact on the occurrence of a BSI, but also the intensity and phase of chemotherapy. Alexander et al and Yacobovich et al similarly reported patients with AML having a higher risk for BSI in comparison to those with other oncological diagnoses.[2,13]

1.4.3 BSI-associated morbidity and mortality

Bloodstream infection contributes significantly to morbidity and mortality in paediatric oncology patients.[1,6] This remains a challenge as newer and more aggressive oncological treatment modalities increase the risk of BSI.

The extent and severity of morbidity and mortality has been shown to vary over time and possibly in relation to other factors.

A mortality prevalence of 8.5% was reported for paediatric haematology-oncology BSI in Johannesburg for the period 1991 to 1995. Increased mortality was recorded in patients who were not commenced on antibiotic therapy shortly after obtaining specimens for blood culture. Gram-negative bacterial infections were also associated with increased mortality risk.[15]

A study in Cairo documented BSI associated mortality of 11.3% during the 2007-2008 period.[1] Viscoli et al in Italy reported a mortality prevalence of 11% within 30 days of the BSI.[6] As this mortality estimate included deaths from the primary oncological disease process and treatment complications, BSI-associated mortality may have possibly been overestimated. More recently, Al-Mulla et al in Qatar reported a mortality prevalence estimate of only 2.2 % for the period 2004 to 2011.[11] This low prevalence may reflect a more

aggressive approach to the investigation and management of BSI at this cancer treatment centre.

The reviewed publications employed a variety of measures for assessing morbidity. Some definitions of BSI-associated morbidity included the persistence of symptoms at day 7 of BSI, the need for ICU admission, the need for CVC removal for persistent BSI or hypotension during the BSI.[1,5,22]

The reported BSI-associated mortality prevalence also varied from cohort to cohort. The composition of cohorts also varied. For example, some cohorts only included paediatric haematology oncology patients, and others cancer patients with CVCs, or patients with a specific type of cancer such as AML. These differences may have influenced the variation of reported BSI associated mortality, which ranged from 2.2% in Qatar to 23% for VGS infection in Taiwan.[22]

1.4.4 Variation of BSI pathogens in relation to cancer diagnosis.

Because of differences in risk factor profile and treatment regimens, it may be anticipated that the pathogens causing BSI may vary depending on the specific type of cancer. However, this literature review showed that only patients with AML experience an increased incidence of BSI caused by specific pathogens.

Johannsen et al in Norway demonstrated that for patients with AML, viridans group *Streptococci* and CONS were the predominant pathogens causing BSI, causing 43% and 9% of BSI, respectively.[20] This infection pattern was attributed to mucositis, antibiotic prophylaxis and the presence of CVCs.[22] Intensive chemotherapy regimens have been reported to increase the risk for VGS BSI, but this was not confirmed by Johannsen who showed that there was no statistically significant difference in the relative occurrence of VGS BSI in AML patients receiving high dose cytarabine and those receiving standard dose cytarabine (P=1.0). Creutzig et al demonstrated more infections during the consolidation phase of treatment with cytarabine than during the more intensive induction chemotherapy phase.[30] This underlines the importance of the other factors that increase the occurrence of VGS BSI in AML patients. Higher incidence of VGS BSI in AML patients was confirmed in studies from Taiwan and Israel.[13,22]

1.4.5 Factors influencing morbidity and mortality in cancer patients with BSI

Specific factors are associated with increased morbidity and mortality in oncology patients with BSI. In an Egyptian study published in 2005, 30.2% of children with BSI experienced persistent fever for ≥ 7 days. Factors associated with this complication included being hospitalized, having intensive chemotherapy, polymicrobial infection, lower respiratory tract infection and persistent neutropaenia. Factors associated with death included being hospitalised, relapse disease, polymicrobial infection and lower respiratory tract infection.[1] Gram-negative BSI has been associated with a significant risk of complications, particularly septic shock.[6,11] Furthermore, infection caused by multidrug resistant pathogens is a major contributor to mortality in oncology patients.[11,31,32]

Although not as common as Gram-positive or Gram-negative infections, fungal BSI causes high morbidity and mortality.[4] A Taiwanese study documented a mortality prevalence of

33.4% in children with *candida* CLABSI, significantly higher than in children with BSI caused by more common pathogens such as *enterobacteriaceae* (7.8%) or CONS (7%). Due to difficulty of treating *candida* CLABSI, CVC removal is necessary in up to 66.7% of patients with this infection. Invasive mycoses particularly have a poor prognosis.[33]

Bloodstream infection-associated mortality is also high in children who have received a HSCT.[6,34]

1.4.6 BSI-associated risk factors

Multiple factors have been demonstrated to increase risk for BSI in paediatric cancer. These risk factors include the presence of neutropaenia, indwelling CVCs, hospitalisation for cancer treatment, HSCT, surgical interventions and relapse of the cancer.

Neutropaenia is a known risk factor for BSI due to the decreased functional capacity of the immune system.[4,19,22] Castagnola in Italy showed that amongst leukaemia patients with a BSI, 80% had neutropaenia. Not only does the presence of neutropaenia have an impact on the occurrence of BSI, but so does the duration of neutropaenia. Patients with prolonged neutropaenia have been shown to have increased risk of BSI.[7,8] This increased risk of BSI in neutropaenia is more pronounced in patients with haematological cancers. In another study in Italy, amongst leukaemia patients 84% of BSI occurred in the presence of neutropaenia.[6] In contrast, amongst patients with solid tumours, 47% of BSI occurred in the presence of neutropaenia. Patients with neutropaenia are susceptible to a specific array of pathogens causing BSI. In France, a study showed that neutropaenic patients with BSI had significant higher rates of oral streptococcal strains, *Enterobacteriaceae* and *Pseudomonas species* on blood culture. This risk is compounded by neutropaenic patients having increased risk of developing HAI.[26] Patients with prolonged neutropaenia are also particularly at risk for fungal infections. For this reason antifungal prophylaxis is recommended in patients where neutropaenia is anticipated to last at least 7-10 days.[35]

In paediatric oncology patients, CVCs are important for the administration of antineoplastic therapy, blood components and nutrition, and recurrent blood sampling.[29] However, CVCs increase the risk of BSI.[28] The risk for BSI is also influenced by the type of CVC. Total external devices (TEDs) such as Hickman CVCs have been shown to pose a higher risk for BSI compared to totally implanted devices (TIDs) such as Port-A-Caths. A prospective study in New Mexico reported that patients with TED CVCs have a 7-fold higher risk of developing BSI in comparison to those with TID CVCs.[17] Similar findings were reported from Israel and Tennessee, USA although the relative difference was lower in those studies.[23,24,28] The spectrum of pathogens causing BSI is also influenced by the type of CVC. In Johannesburg, patients with Hickman catheters were shown to preferentially develop Gram-negative sepsis. However, Hickman CVCs was the only type of CVC used in the study patients. In Israel, patients with different CVC types were shown to have a statistically significant difference in infection pattern in relation to the CVC type. Patients with Hickman lines predominantly had Gram-negative catheter-associated BSIs whilst patients with TID CVCs mainly had Gram-positive infections.[23] A similar pattern was reported in another Israeli study.[24]Fungal BSI is also associated with indwelling CVCs particularly with TED CVCs.[15,19]

Oncology patients tend to require indwelling CVCs for lengthy periods of time whilst under treatment, including periods when neutropaenia is absent. During the maintenance phase of

chemotherapy when neutropaenia is usually absent CVCs remain a major risk factor for the development of BSI.[19] Overall, the risk for the development of CVC-associated BSI was shown to be highest soon after CVC insertion possibly due to co-existing neutropaenia which develops after the commencement of chemotherapy.[17] Another contributing factor could be sub-optimal management of indwelling CVC by the family during the initial period of chemotherapy. Although neutropaenia is a known risk factor for BSI, other risk factors should be considered. For example, in a prospective multicentre study completed in Italy there was a difference in the occurrence of CVC-associated BSI between patients with neutropaenia and without neutropaenia. Here the proportion of catheter-associated infections was 20% in neutropaenic patients in comparison to 55% in non-neutropaenic patients.[6] This difference was statistically significant. This underlines the morbidity associated with CVCs, even in the absence of neutropaenia.

Patients receiving chemotherapy or other therapies for cancer require repeated hospitalisation. Hospitalisation *per se* is a risk factor for HAI. In a study in France 64% of BSIs were hospital-acquired.[26] A study in Egypt showed that HAIs were significantly more frequent in neutropaenic patients than non-neutropaenic patients. Hospitalisation was shown to increase risk of catheter-associated BSI (CABSI) in Israel.[22] However; an American study reported that the majority of CABSIs occurred in ambulatory patients.[5] These contrasting findings may be related to different local management practices and differences in the length of hospitalisation in patients with CVCs on chemotherapy. The definition used for CABSI and CLABSI in the published literature was the same as the definition of CLABSI proposed by the Center for Disease Control and Prevention.[36]

Depending on the primary cancer diagnosis and the response to therapy, some patients may need HSCT. Haematopoietic stem cell transplantation increases the risk for BSI particularly in the pre-engraftment period.[2] In Israel, patients were shown to have a 2-fold increased risk of developing a CABSI following HSCT.[23] Similarly, in another study post-HSCT patients were shown to have a high incidence of BSI, even higher than in myeloid leukaemia.[13] However, in this study only 6 patients underwent HSCT, thus clear conclusions cannot be deduced. Increased risk of BSI post-HSCT has been shown not only to increase bacterial BSI but also invasive fungal infections.[37] This increased risk for BSI following HSCT is higher post-allogeneic HSCT than with autologous stem cell transplant.[34]

The age at diagnosis of paediatric oncology patients varies widely and is influenced by the specific cancer diagnosis. Age may influence the risk for BSI. However, in studies completed in France and Israel, younger age was not shown to be associated with BSI.[13,28]

1.4.7 Antibiotic susceptibility patterns

Patients with cancer may mount a sub-optimal immune response to infection. Hence it is important to optimise antibiotic choices including empiric antibiotic therapy for BSI, particularly in settings where resistance to commonly used antibiotics is frequent. For example, Egyptian studies have documented high frequencies of antibiotic resistance among pathogens causing BSI in children with oncological conditions.[1,12] An Israeli study documented higher antibiotic resistance among Gram-negative pathogens, particularly *Pseudomonas, Klebsiella* and *Acinetobacter* isolates, although these pathogens were not associated with increased mortality.[24]

Resistance has also been documented among viridans group streptococci. A Norwegian study showed that 40% of viridans group streptococci causing BSI in cancer patients between 2004 and 2011 were resistant to penicillin.[20] Similarly in Taiwan 56% VGS isolates cultured from haematology-oncology patients between 1998 and 2004 were resistant to penicillin.[22] Multi-drug resistance is a particular concern. In Qatar a study of BSI in oncology patients on chemotherapy showed that 28.4% of bacterial isolates were multidrug resistant. Multi-drug resistant pathogens caused the BSI in 3 of 4 children who died as a consequence of their BSI .[11]

1.5 Research gaps:

This literature review showed that BSI is a frequent occurrence in paediatric oncology patients and an important cause of morbidity and mortality. Increased risk for BSI may be due to the cancer itself, chemotherapy, CVCs, hospitalisation and infections of multi-drug resistance.

However, from a South African and sub-Saharan African perspective, the following gaps in knowledge were identified in this literature review.

1- Paucity in publications from South Africa and the rest of Africa. Only 1 article was from South Africa was identified, published approximately 20 years ago.
2- The majority of the publications reviewed did not analyse the antibiotic susceptibility pattern of the pathogens causing BSI and there is no literature on the antibiotic susceptibility patterns of BSI in paediatric oncology in South Africa.
3- Morbidity and mortality was not consistently described. When morbidity was described, there was a marked variation in how morbidity was defined. Common complications of BSI in oncology patients have not been adequately documented in African settings.
4- A description of fungal BSI was not included in many studies. Most articles focussed exclusively on BSI caused by bacterial pathogens.
5- Limited study of the relationship between the phase of chemotherapy or bone marrow status and BSI risk or outcome after BSI.
6- Limited study of the relationship between non-chemotherapeutic interventions such as surgery and radiotherapy and the risk of BSI in paediatric oncology patients.
7- Limited understanding of the impact of HIV infection on the risk and outcome of BSI.

The following study on the epidemiology of BSI at RCWMCH aims to bridge some of these knowledge gaps, by exploring the spectrum of bacterial and fungal pathogens causing BSI, the morbidity and mortality of BSI and the antibiotic susceptibility patterns of pathogens causing BSI in children with an underlying oncological condition, treated at a South African paediatric referral hospital.

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Chapter 2: Publication-ready manuscript

Blood stream infections in Oncology patients at Red Cross War Memorial Children`s Hospital, Cape Town

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2.1 Abstract

Background: Infections cause significant morbidity and mortality in children with cancer, which may be related to the cancer or treatment received. There is paucity of data on the epidemiology of bloodstream infection (BSI) in sub-Saharan Africa. To address this knowledge gap, this study was conducted at Red Cross War Memorial Children`s Hospital (RCWMCH) in Cape Town, South Africa.

Methods: A retrospective cohort study was conducted at the haematology-oncology unit of RCWMCH. All positive blood cultures from RCWMCH oncology patients taken between 1 January 2012 and 31 December 2014 were retrieved to identify patients who had BSI.

Results: From 436 positive blood culture results, 150 BSI episodes were identified amongst 89 patients; 49.1% of the culture isolates were Gram-positive bacteria, 41.6 were Gram-negative bacteria and 9.3% were fungal. Coagulase Negative *Staphylococcus* and *Viridans Group Streptococcus* were the most common Gram-positive isolates, and *Escherichia coli* and *Klebsiella species* the commonest Gram-negative isolates. The majority of BSI episodes occurred in patients with haematological malignancies (74%), in the presence of severe neutropaenia (76.4%) and whilst on or following chemotherapy (88%). Complications occurred in 14% of the BSI episodes. Fungal infections had the highest prevalence of complications (21.4%). Three children died during BSI as a result of multidrug resistant isolates, giving a case-fatality rate of 2%.

Conclusion: BSI in our patients are mainly caused by Gram-positive bacteria and associated with a low case-fatality rate. The results of this study are consistent with worldwide experience of BSI in paediatric oncology patients.

[245 words]

2.2 Introduction

Infections are a significant contributing factor to morbidity and mortality in paediatric oncology.[1,2] Immunity may be compromised by cancer itself or therapeutic interventions. Patients with haematological malignancies and cancers with bone marrow infiltration are at increased risk of infection. Bone marrow suppression with neutropaenia and/or lymphopaenia may occur as a result of chemotherapy and radiotherapy rendering the innate and adaptive immune systems dysfunctional.[3,4,5] Such patients easily develop opportunistic infection.[6]

With advances in cancer treatment, more intensive treatment regimens have been introduced leading to increased cure rates but also increased infection risk. Hence the high rate of infection in patients with acute myeloid leukaemia (AML) where very myelosuppressive chemotherapy causes profound and prolonged neutropaenia.[7]

Cancer patients have increased exposure to pathogens because of indwelling vascular access devices (VAD) and frequent hospitalisation. This places them at risk for hospital-acquired infections (HAI) which carry high morbidity as they are frequently caused by multi-drug resistant (MDR) organisms.[8]

A number of studies have described the epidemiology of bloodstream infections (BSI) in paediatric oncology worldwide. There is variation in the common pathogens, BSI risk factors, extent of morbidity or mortality and antibiotic susceptibility patterns as a result of [1,2,7]environmental factors, local cancer treatment regimens or antibiotic selection pressure.

There is a marked paucity of data on BSI amongst paediatric oncology patients in low and middle income countries (LMIC). At the research site, BSI is an important contributor to hospitalisation.[9] No studies have been done in children with cancer at this site, and only one such study has been completed in South Africa.[2] Therefore, we investigated the epidemiology of BSI in paediatric oncology patients at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa, including the spectrum of pathogens, their antimicrobial susceptibilities and clinical outcomes.

2.3 Materials and methods

2.3.1 Study design and setting

This retrospective cohort study was conducted in the 17-bed haematology-oncology unit at RCWMCH in children with cancer who developed culture-confirmed BSI between 1 January 2012 and 31 December 2014. This oncology unit serves approximately 50% of the children less than 15 years of age with cancer in the Western Cape Province and selected referrals from the Eastern Cape Province, diagnosing and treating between 100 and 130 children annually.

2.3.2 Study population

Blood culture specimens are collected in neutropaenic patients if they have an axillary temperature >37.5° Celsius (C) on at least two occasions, or an axillary temperature > 38ºC on one occasion and/or have clinical features suggestive of infection. In non-neutropaenic patients, blood culture specimens are collected if the axillary temperature is ≥38°C and/or there are clinical features suggestive of infection. Culture specimens are obtained aseptically. A second culture is obtained if the axillary temperature rises over 38ºC on a second occasion,

and then the patients are only cultured again if the clinical condition deteriorates. For this study, culture specimens had been collected aseptically either by peripheral venepuncture or from central lines. In patients with suspected BSI and VADs *in situ*, blood cultures were not routinely obtained from both the VAD and a peripheral vein. During the study period, Piperacillin-tazobactam and Amikacin was the empiric antibiotic regimen for suspected BSI in children with neutropaenia, whilst empiric Ceftriaxone was prescribed in the absence of neutropaenia. Patients at RCWMCH oncology unit with haematological malignancies routinely receive Cotrimoxazole as antibiotic prophylaxis for the duration of their treatment. Patients who are expected to have neutropaenia for longer than 7 days are given Fluconazole prophylaxis. Quinolones are not used for prophylaxis.

During the study period, 3050 blood culture specimens were collected and processed by the National Health Laboratory Services (NHLS) microbiology laboratory based at Groote Schuur Hospital; 436 of these blood cultures yielded positive results. Organisms were stratified into pathogens and contaminants. Coagulase-negative staphylococci (CONS), *Staphylococcus epidemidis, Bacillus spp*. *Micrococcus spp*. and Coryneform bacteria were regarded as contaminants and excluded from the analysis unless they were cultured on two or more independent blood culture specimens within a 48-hour period, in which case they were regarded as pathogens.[10] This approach was used irrespective of whether blood culture specimens were obtained by peripheral venepuncture or a central line. Isolates of *Enterococcus species* and Viridans group streptococcus (VGS) were considered pathogenic when isolated whilst there was a fever at the time of blood culture.

16 positive blood cultures were excluded because they were obtained from children with non-oncological haematological diseases. A further 77 were excluded because they yielded contaminants. The remaining 343 positive blood culture results were attributed to 150 BSI episodes in 89 oncology patients. There were 173 different culture isolates causing these 150 BSI episodes. Amongst these 89 children, 56 had one episode of BSI and the remaining 33 children had 2 or more episodes during the study period. Recurrent BSI was defined as the isolation of the same or different organism on blood culture more than 14 days after the initial or previous BSI.

Haematological tumours, solid tumours and central nervous system (brain) tumours are the 3 main types of paediatric cancer. Research has shown variation in the incidence of BSI in relation to tumour type, with haematological cancer having a higher incidence.[1] Amongst haematological cancers, AML patients are at a higher risk for BSI.[6] To investigate variation in the occurrence of BSI, the study population was stratified into the three main types, and the haematological malignancies further classified into AML, acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) sub-types.

2.3.3 Data Collection

Clinical data relating to each BSI were extracted from patient hospital records and transferred to a study-specific data collection sheet. The date of each BSI, age, gender, weight, HIV status, temperature at time of blood culture, cancer diagnosis, details of prior or current chemotherapy, surgery or radiotherapy, presence and type of VAD, absolute neutrophil count (ANC) were documented. Microbiological results were obtained from the NHLS database. Antimicrobial therapy, complications and outcome of each BSI were documented.

2.3.4 Microbiological procedures

Microbiological testing of blood culture specimens was completed at the Groote Schuur Hospital NHLS microbiology laboratory using the BACTEC^{TM} 9240 automated blood culture system (Becton Dickinson, Sparks, Maryland). If growth of any pathogen was detected, standard biochemical, disk diffusion and gradient diffusion antibiotic susceptibility tests were used to evaluate gram-positive organisms. Gram-negative organisms were identified and antibiotic susceptibility tested after direct inoculation of the bacterial colonies into the automated Vitek®2 system [bioMérieux, Inc., France] using Vitek®2 ID-GNB and AST-N064 cards. Where necessary, repeat testing was done on bacterial colonies subcultured onto agar plates, using either Vitek®2, disk diffusion or gradient diffusion E-test (bioMérieux, Marcy l'Etoile, France) methods. Fungal isolates identified on gram stain were inoculated onto sabouraud dextrose agar. Identification and susceptibility testing of fungi were carried out with the Vitek^{R} 2 system using YST identification and AST-YS07 cards. Susceptibility results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria for 2012 – 2014.[11]

The following definitions were used:

Nutritional status: moderate and severe underweight were defined as weight-for-age z score (WAZ) between -2 and -3 standard deviations (SD) below the median World Health Organisation (WHO) growth reference standards, and a WAZ < -3 SD respectively.

HIV infection: HIV infection was diagnosed in a child <18 months old using HIV PCR test and in a child $\geq$ 18 months using serological tests as per guidelines from Schneider et al.[12]

Neutropaenia: The presence of an ANC of < 1500 x 10^9/ L was regarded as neutropaenia. An ANC of < 500 x 10^9/ L was classified as severe neutropaenia.[13] Although we employed this definition, it may differ from the definition used in other published studies.

Polymicrobial BSI: BSI episode whereby 2 or more different isolates were grown from the same blood culture sample.

Venous Access Device (VAD): An indwelling venous catheter that was inserted into the central venous system with the catheter tip positioned within the superior/inferior vena cava or right atrium, including Hickman, Port-A-Cath and central venous pressure (CVP) catheters.[14]

2.3.5 Data analysis

All data were entered anonymously into a dedicated Microsoft Access 2013 version 15.0 database. All analyses were performed using STATA IC version 14.1 (College Station, Texas). Frequency tables were used to summarise the baseline characteristics at the time of BSI. Baseline characteristics at time of BSI are presented as proportions with percentages if categorical and compared across cancer groups using Fisher's exact test. Continuous data normally distributed are summarized using mean and standard deviation (SD) and compared across groups using Analysis of Variance (ANOVA) test. Skewed continuous data are summarized as medians with inter-quartile range (IQR) and compared across groups using Kruskal–Wallis test. Statistical significance was considered at a two-sided α-level of 0.05

2.3.6 Ethical considerations

The study was completed in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, reference number: HREC 021/2015. Informed consent was not obtained from individual patients or caregivers because the data was collected retrospectively. Patient details were anonymised before data analysis.

2.4 Results

2.4.1 Prevalence of bloodstream infection

During the study period, 332 cancer patients were managed in the oncology unit. BSI was more common amongst children with AML than those with other tumours, 27/33 (81.8%) vs 62/299 (20.8%), risk ratio 3.9, 95% confidence interval: 3.0 – 5.2. Of all children who experienced BSI episodes, those with AML experienced close to twice the BSI episodes per child compared to those with brain tumours (Table 1).

2.4.2 Baseline patient characteristics

Table 2 summarises the patient characteristics at the time of BSI and disaggregates this information according to cancer type and haematological cancer sub-type. The mean age at the time of BSI was higher among patients with haematological malignancies compared to those with other cancers but this difference was not statistically significant. Overall, 23.3% (35/150) BSI episodes were associated with moderate or severe underweight-for-age, and of 108 episodes in which HIV status was documented, two (1.9%) occurred in patients with HIV infection. Severe neutropaenia was significantly more frequent in BSI episodes in patients with AML. Prolonged neutropaenia for at least 7 days was present in 40.5% of BSI episodes and significantly more frequent in BSI episodes with ALL or AML compared to other tumours.

Most BSI episodes occurred during the induction and consolidation phases of chemotherapy for haematological malignancies, and during the continuous phase of solid tumour chemotherapy. For the timing of BSI in relation to chemotherapy, 88% (132/150) occured within 30 days of receiving chemotherapy. A VAD was *in situ* at the time in 68% (102/150) of the BSI episodes, and was more frequent in episodes associated with AML and NHL. Amongst patients with VAD, 44.7% (59/132) experienced at least one BSI compared to 15% (30/200) in patients without VAD, $p<0.01$.

2.4.3 Spectrum of microorganisms

The majority of the 150 BSI episodes had no identifiable clinical focus of infection (Table 2) Of these episodes, 129 (86%) were caused by a single bacterial or fungal isolate (monomicrobial infections) i.e. 61 were caused by gram positive bacteria, 54 by gram negative bacteria, and 14 by fungal species. The remaining 21 (14%) BSI episodes were polymicrobial infections. Nineteen and two polymicrobial BSI were caused by 2 and 3 pathogens, respectively. These polymicrobial infections occurred amongst 20 patients, with 1 patient experiencing two polymicrobial BSI.

The distribution of the 173 BSI-causing microorganisms is summarised in Table 3. Gram-positive bacteria, Gram-negative bacteria and fungi were responsible for 49.1%, 41.6 % and 9.3% of the isolates, respectively. CONS, VGS and enterococcus species were the most frequently isolated Gram-positive bacteria. Of 72 Gram-negative bacterial isolates, 51 (70.8%) belonged to the *enterobacteriaceae* family. All fungal isolates were *Candida species*. VGS was the commonest isolate in AML patients with BSI whilst CONS was the commonest isolate in ALL and NHL patients with BSI.

Of the 121 BSI associated with neutropenia, 52 (43%), 44 (36.4%), and 10 (8.3%) were single-isolate Gram-positive bacterial, Gram-negative bacterial and fungal infections respectively, and the remaining 15 (12.4%) BSIs were polymicrobial infections. By contrast, of the 23 episodes without neutropaenia, 8 (34.8%), 6 (26.1%), and 3 (13%) were single-isolate Gram-positive bacterial, Gram-negative bacterial, and fungal infections, respectively, and 6 (26.1%) were polymicrobial infections. The proportion of single-isolate Gram-positive bacterial infections was higher when neutropaenia was present, but this was not statistically significant, p=0.46. The proportion of CONS was similar in BSI episodes associated with neutropaenia compared to episodes not associated with neutropaenia [31/121 (25.6%) *vs* 6/23 (26.1%), p=1.0]. Furthermore, while the proportion of VGS was higher in BSI episodes associated with neutropaenia, this difference was not statistically significant, [(20/121 (16.5%) vs 1/23 (4.31%), p=0.2)].

For patients with a VAD compared to those without VAD, single-isolate Gram-negative bacterial infections were significantly less common [31/102 (30.4%) *vs* 23/48 (47.1%), p= 0.04]. Single-isolate Gram-positive bacterial, polymicrobial and single-isolate fungal infections were more common but not significantly different in BSI episodes with a VAD [44/102 (43.14%) *vs* 17/48 (35.4%), p=0.48], [16/102 (15.7%) *vs* 5/48 (10.4%), p=0.46], [11/102 (10.8%) *vs* 3/48 (6.3%), p=0.55], respectively. CONS occurred in higher proportion amongst BSI episodes with a VAD compared to episodes without a VAD [30/102 (29.4%) vs 7/48 (14.6%), p=0.07] whilst *Escherichia coli* occurred less frequently [7/102 (6.9%) vs 9/48 (18.8) (p=0.04)]

The distribution of infection types in relation to the different VADs that was present at the time of BSI is summarised in Figure 1. Of 102 BSI episodes with a VAD *in situ*, 58 (56.9%), 37 (36.2%) and 7 (6.9%) had Hickman, Port-A-Cath and CVP catheters, respectively. Single-isolate Gram-positive bacterial infections predominated in the presence of Hickman and Port-A-Cath VADs. During the study period, amongst all 332 oncology patients managed in the haematology-oncology unit, 124 (37.4%) required VADs. Amongst these 124 patients, 65 (52.4%), 47 (37.9%) and 12 (9.7%) had Hickman, Port-A-Cath and CVP catheters, respectively.

During the study period, 25 oncology patients were being followed up by the oncology unit after haematopoietic stem cell transplantation (HSCT). Seven BSI episodes occurred in 7 of these patients. Of these 7 BSI, 42.9% (3/7) were caused by Gram-positive bacteria, 42.9% (3/7) were caused by Gram-negative bacteria and 14.2% (1/7) were polymicrobial in aetiology. Amongst the 143 BSI not preceded by HSCT Gram-positive bacteria, Gram-negative bacteria, polymicrobial growth and fungi caused 40.6% (58/143), 35.7% (51/143),

14% (20/143) and 9.8% (14/143), respectively. There was no difference in the spectrum of pathogens causing BSI in relation to HSCT (p=1.0)

Radiotherapy preceded 8% (12/150) of the BSI episodes. The difference in causative microorganisms when comparing BSI episodes preceded by radiotherapy and those not preceded by radiotherapy was minimal.

2.4.4 Antibiotic and antifungal susceptibilities of BSI isolates

Table 4 shows the antimicrobial susceptibility patterns of the bacterial and fungal isolates causing BSI. Low carbapenem resistance was documented amongst the Gram-negative bacteria. All fungal isolates were susceptible to amphotericin B. The median time to effective antimicrobial therapy was 0 (IQR 0-1) days in the 138 isolates where this information was known. In 61.6% (85/138) of these isolates, the empiric antibiotic therapy initiated at the time of blood culture acquisition was effective. Amongst the 137 bacterial isolates obtained from BSI episodes with neutropenia, 46 were tested for Pipericillin-tazobactam and Amikacin susceptibility and 13/46 (28.3%) were resistant to the standard empiric Pipericillin-tazobactam and Amikacin regimen.

2.4.5 Outcome

At least one complication including death occurred in 14% (21/150) of all BSI episodes. The more frequent complications were septic shock in 8% (12/150), respiratory failure in 2.7% (4/150), renal failure in 2% (3/150) and multi-organ failure in 1.3% (2/150). Other complications included GIT infection, bleeding or perforation (4), abscesses (2), coagulopathy or thrombosis (2) and empyema or pneumothorax (2). Complications occurred in 21.4% (3/14), 20.4% (11/54), 9.5% (2/21) and 8.2% (5/61) of fungal, Gram-negative bacterial, polymicrobial and Gram-positive bacterial BSI episodes respectively, p=0.19. Treatment in the intensive care unit (ICU) was required in 7.3% (11/150) of BSI episodes. Bloodstream infection episodes caused by isolates which were sensitive to the empiric antibiotic regimens were associated with fewer complications than episodes caused by resistant isolates, 9/85 (10.6%) *vs* 7/53 (13.2%), p=0.79,and fewer deaths, 2/85 (2.4%) *vs* 2/53 (3.8%), p=0.64. These differences were not statistically significant.

During the study, there were 3 deaths from BSI giving a case-fatality rate (CFR) of 2%. Two of the deaths occurred in children with BSI caused by extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates. One of these two children developed the *Klebsiella pneumoniae* BSI day 53 post-HSCT. The third death occurred in a patient with a polymicrobial BSI caused by Vancomycin-resistant *Enterococcus faecium* and *Streptococcus viridans*. This patient also had mucormycosis which was diagnosed on histological tissue examination of small and large bowel specimens collected on laparotomy. All 3 patients died in ICU, and on transfer to ICU, 2 had multi-organ failure and 1 had respiratory failure.

2.5 Discussion

Since the 1970s an increasing proportion of Gram-positive bacteria has been noted as the cause of BSI in paediatric oncology.[3,4,6,15,16] Similarly, in the present study Gram-positive bacteria particularly CONS and VGS were the predominant causes of BSI. A previous hospital-wide study at RCWMCH showed that Gram-negative bacteria are the main causes of

BSI.[9] The susceptibility to Gram-positive bacteria among oncology patients may relate to several factors including the high prevalence of neutropaenia, intensive chemotherapy regimens, mucosal barrier defects and prophylactic antimicrobial use during periods of immune vulnerability. Neutropaenia disables the immune system, increasing the risk of infection and alters the spectrum of organisms that cause infection.[7,17] Intensive chemotherapy increases this risk by causing neutropaenia and defects in mucosal barriers such as mucosits.[3,18] Defects in mucosal barriers of the gastrointestinal tract increase the risk for infection by bacteria that normally colonise the gastrointestinal tract.[18,19]

Patients with haematological cancers at RCWMCH, particularly AML and ALL, were shown to have higher BSI frequency. Previous studies have shown that AML patients have higher risk of BSI than other malignancies, and are particularly susceptible to infections with VGS as is also demonstrated at RCWMCH.[7,20,21] At RCWMCH, VGS is considered a pathogen if isolated from a single blood culture specimen in a febrile child suspected of having BSI. The prevalence of VGS BSI in AML was similar in the induction and consolidation chemotherapy phases in our study. Similar findings were reported in Denmark.[18]

In Johannesburg and Doha the majority of BSI occurred in the absence of neutropaenia.[2,3] Some studies have however showed that neutropenia is a significant risk factor for BSI.[7,8,22,23] In the present study, the majority of BSI (76.4%) occurred in the presence of severe neutropaenia.

The presence of a VAD is an important risk factor for BSI. Furthermore, CONS was a frequent cause of BSI in patients with a VAD, whereas Gram-negative infections predominated in those without a VAD.[17,24,25] In the Johannesburg study, more Gram-negative bacterial infections were reported with Hickman catheters whilst Gram-positive infections were more common in totally implantable VAD.[2] A study in Ohio demonstrated increased VAD associated BSI incidence in the inpatient setting in comparison to the ambulatory setting. In Ohio, different types of pathogens were identified as the cause of BSI in relation to different VAD type. The pathogen type also varied whether the patient was an inpatient or not.[26] In the present study, Gram-positive infections were commoner in Hickman and Port-A-Cath types of VAD. This difference may be due to local practices. The current study at RCWMCH did not look into whether the first signs and symptoms of the BSI in patients with a VAD developed as an inpatient or outpatient. At RCWMCH, Gram-negative infections occurred significantly less frequently in patients with a VAD. By contrast, CONS occurred more commonly in the presence of a VAD. This suggests that the presence or absence of a VAD has an effect on changing patterns of pathogens causing BSI in patients.

Chemotherapy is also a risk factor for BSI because of neutropaenia, prolonged or repeated hospitalisation and the presence of VAD.[3,27] Patients with prolonged hospitalisation may be colonised by hospital based pathogens and about 25% of them develop BSI.[28] A study in cancer patients in France reported that 66.2% of BSI episodes were nosocomial.[29] At RCWMCH, 88% of BSI episodes occurred within 30 days of chemotherapy, especially during the induction phase of haematological malignancy chemotherapy (35.3%) and the continuation phase of solid tumour chemotherapy (29.3%). Young age is another risk factor for BSI.[20]

The antibiotic susceptibility results suggest that the combination of Piperacillin-tazobactam plus Amikacin is an effective empiric antibiotic option for the majority of Gram-positive and Gram-negative infections and may continue to be used in the haematology-oncology unit in children with febrile neutropaenia. However, the antimicrobial susceptibility results on the Gram-positive isolates in our study were incomplete, and the available evidence in Table 4 show that a high proportion of the Gram-positive isolates are susceptible to Vancomycin. Hence, Vancomycin should be considered for patients who do not show a satisfactory clinical response to empiric therapy.

The high proportion of Gram-negative isolates that were susceptible to the carbapenems is reassuring and reflects the tight regulation of their usage in the unit. No resistance among fungal isolates to amphotericin B is also reassuring, especially when required for empirical treatment of children with severe infections.

In the present study, complications were more frequent in fungal BSI than bacterial BSI. Although fungal infections are often lethal, they are less frequent than bacterial infections.[7] The CFR of 2% was low in comparison to other studies. A study conducted in Johannesburg before 2000 documented a CFR of 8.5%, whereas a recent study conducted in Qatar recorded a CFR of 2.2%.[2,3] Furthermore, a study of infectious complications in children with AML and Down syndrome reported no deaths from bacterial or fungal infections.[30] Low mortality at RCWMCH may reflect an aggressive approach to monitoring for BSI in oncology patients, lower resistance rates of microbial isolates and the short median time to initiating effective antimicrobial therapy of 0 days.

Study strengths and limitations

A strength of this study is that it is one of few sub-Saharan African studies to describe bloodstream infections in childhood cancer. Although the results of this study are not generalizable to other paediatric haematology-oncology services in sub-Saharan Africa, it provides an understanding of the spectrum of organisms causing BSI and the outcome of BSI in an African context. The results highlight low mortality as a result of effective management of both the patients and the existing antimicrobial resources. Due to the retrospective design, there are limitations in the completeness of the clinical and laboratory data, and antimicrobial susceptibilities were incompletely evaluated. Because of low numbers of children with HIV infection, it was not possible to explore the effect of HIV infection on the course and outcome of BSI in this study, Furthermore, few deaths meant that risk factors for mortality were not evaluated by univariate and multivariate analyses.

2.6 Conclusion

BSI in our paediatric haematology-oncology unit is mainly caused by Gram-positive bacteria, in keeping with worldwide experience. Patients with haematological cancer, particularly AML were demonstrated to have the higher risk of BSI. Low levels of carbapenem resistance among Gram-negative isolates are encouraging. A prospective study involving multiple paediatric oncology centres is required to address limitations identified in the present study and provide a more comprehensive understanding of BSI in children with cancer in South Africa and sub-Saharan Africa.

2.7 Author contributions

Tisungane Mvalo collected the data from the RCWMCH patient files and the NHLS results database for the investigated cases and wrote the manuscript. Alan Davidson proposed the title and objectives of the study. Brian Eley provided guidance on study literature review, data analysis and manuscript development with Alan Davidson. Colleen Bamford provided the list of retrieved blood cultures from the Haematology-Oncology unit from RCWMCH to identify cases of BSI. Christopher Stanley and Maganizo Chagomerana performed the primary data analysis and described its methodology. All authors reviewed and approved the final draft.

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2.10 Tables, figures and legends

Table 1: The distribution of cancer and the frequency of BSI amongst cancer patients in the oncology unit during the period 2012-2014

Parameter	AML n (%)	ALL n (%)	NHL n (%)	Solid tumour n (%)	Brain tumour n (%)	Total n (%)
Number (%) of cancer patients	33 (9.9%)	62 (18.7%)	39 (11.7%)	126 (38%)	72 (21.7%)	332
Number (%) of cancer patients with BSI	27/33 (81.8%)	23/62 (37.1%)	12/39 (30.8%)	23/126 (18.3%)	4/72 (5.6%)	89/332 (26.9%)
Number (%) of BSI episodes	53 (35.3%)	41 (27.3%)	17 (11.3%)	35 (23.3%)	4 (2.7%)	150
Number of BSI episodes per patient with BSI	1.96	1.78	1.42	1.52	1.0	1.69

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; NHL, non-Hodgkin lymphoma, BSI, bloodstream infection

Table 2: Characteristics at the time of BSI

Parameter Number of BSI episodes	AML N=53	ALL N=41	NHL N=17	Solid tumour N=35	Brain tumour N=4	Total N=150	P-value
Gender, n (%)							
Male	26 (49.1)	23 (56.1)	10 (58.8)	21 (60)	1 (25)	81 (54)	0.65 *
Female	27 (50.9)	18 (43.9)	7 (41.2)	14 (40)	3 (75)	69 (46)	
Age category n (%)							
< 5 years	30 (56.6)	23 (56.1)	8 (47.1)	26 (74.3)	3 (75)	90 (60)	
5-15 years	23 (43.4)	18 (43.9)	9 (52.9)	9 (25.7)	1 (25)	60 (40)	0.27 *
Mean age ± SD in years	4.9 ± 3.6	5.1 ± 3.8	5.9 ± 3.5	4.1 ± 3.3	3.0 ± 1.9	4.8 ± 3.6	0.37 ǂ
Median (IQR) weight–for-age Z -score	-0.9 (-1.8, -0.2)	-0.6 (-1.1, -0.1)	-1.6 (-2.3, -1.4)	-1.1 (-2.8, -0.1)	-1.4 (-3.0, -0.6)	-1.0 (-1.9, 0.0)	0.08 Ψ
Weight-for-age Z-score category, n (%)							
Moderate under-weight-for-age, n (%)	4 (7.5)	3 (7.3)	4 (23.5)	6 (17.1)	0 (0)	17 (11.3)	
Severe under-weight-for-age, n (%)	5 (9.4)	0 (0)	4 (23.5)	8 (47)	1 (25)	18 (12)	0.44 *
ANC at time of BSI, n (%)							
<500 cells/µL	46 (88.5)	31 (79.4)	14 (82.3)	17 (51.5)	2 (66.7)	110 (76.4)	0.01 *
500 – 1500 cells/µL	2 (3.8)	4 (10.3)	0 (0)	5 (15.2)	0 (0)	11 (7.6)	
> 1500 cells/µL	4 (7.7)	4 (10.3)	3 (17.7)	11 (33.3)	1 (33.3)	23 (16)	
Neutropaenia duration, n (%)							
< 7days	23 (47.9)	15 (42.9)	12 (85.7)	20 (90.9)	2 (100)	72 (59.5)	
≥ 7days	25 (50.1)	20 (57.1)	2 (14.3)	2 (9.1)	0 (0)	49 (40.5)	<0.01 *
Isolate count per BSI							
Monomicrobial	48 (90.6)	35 (85.4)	12 (70.6)	30 (85.7)	4 (100)	129 (86)	
Polymicrobial	5 (9.4)	6 (14.6)	5 (29.4)	5 (14.3)	0 (0)	21 (14)	0.36 *
Bone marrow status							
In remission	31 (58.5)	17 (41.5)	2 (11.8)	3 (8.6)	0 (0)	53 (35.3)	
Not in remission	10 (18.9)	11 (26.8)	0 (0)	6 (17.1)	0 (0)	27 (18.0)	<0.01 *
Not investigated	12 (22.6)	13 (31.7)	15 (88.2)	26 (88.3)	4 (100)	70 (46.7)	
Chemotherapy phase at time of BSI							
Induction	30 (56.6)	21 (51.2)	2 (11.8)	0 (0)	0 (0)	53 (35.3)	
Consolidation	19 (35.8)	8 (19.5)	0 (0)	0 (0)	0 (0)	27 (18)	
Intensification	0 (0)	4 (9.8)	0 (0)	0 (0)	0 (0)	4 (2.7)	
Maintenance	0 (0)	1 (2.4)	0 (0)	3 (8.6)	0 (0)	4 (2.7)	
Continuous	1 (1.9)	1 (2.4)	14 (82.3)	24 (68.6)	4 (100)	44 (29.3)	
No chemotherapy	3 (5.7)	6 (14.6)	1 (5.9)	8 (22.8)	0 (0)	18 (12)	
Clinical focus of infection							
Yes	21 (39.6)	11 (26.8)	11 (64.7)	12 (34.3)	0 (0)	55 (36.7)	
No	32 (60.4)	30 (73.2)	6 (35.3)	23 (65.7)	4 (100)	95 (63.3)	0.04 *
Venous access device							
Present	47 (88.7)	19 (46.3)	15 (88.2)	20 (57.1)	1 (25)	102 (68)	
Absent	6 (11.3)	22 (53.7)	2 (11.9)	15 (42.9)	3 (75)	48 (32)	<0.01 *
Recorded temperature							
>35.5-37.9 degrees Celsius	9 (20.5)	2 (12.5)	0 (0)	1 (9.1)	0 (0)	12 (16)	0.87 *
>=38 degrees Celsius	35 (79.5)	14 (87.5)	3 (100)	10 (90.9)	1 (100)	63 (84)	
Surgical procedure within last 3 months							
Yes	43 (81.1)	16 (39)	15 (88.2)	16 (45.7)	2 (50)	92 (61.3)	
No	10 (18.9)	25 (61)	2 (11.8)	19 (54.3)	2 (50)	58 (38.7)	<0.01 *
HIV status							
Infected	0 (0)	0 (0)	2 (11.8)	0 (0)	0 (0)	2 (1.3)	
Uninfected	38 (71.7)	27 (65.9)	14 (82.3)	24 (68.6)	3 (75)	106 (70.7)	0.07 *
Not documented	15 (28.3)	14 (34.1)	1 (5.9)	11 (31.4)	1 (25)	42 (28)	

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ANC, absolute neutrophil count; NHL, non hodgkin lymphoma; VAD, venous access device

Bone marrow status was determined from the last haematological examination of bone marrow aspirate and or trephine biopsy performed on the patient.

*** Fischer`s exact test; ǂ ANOVA test; Ψ Kruskal-Wallis test**

Table 3: Distribution of blood culture isolates in relation to malignancies

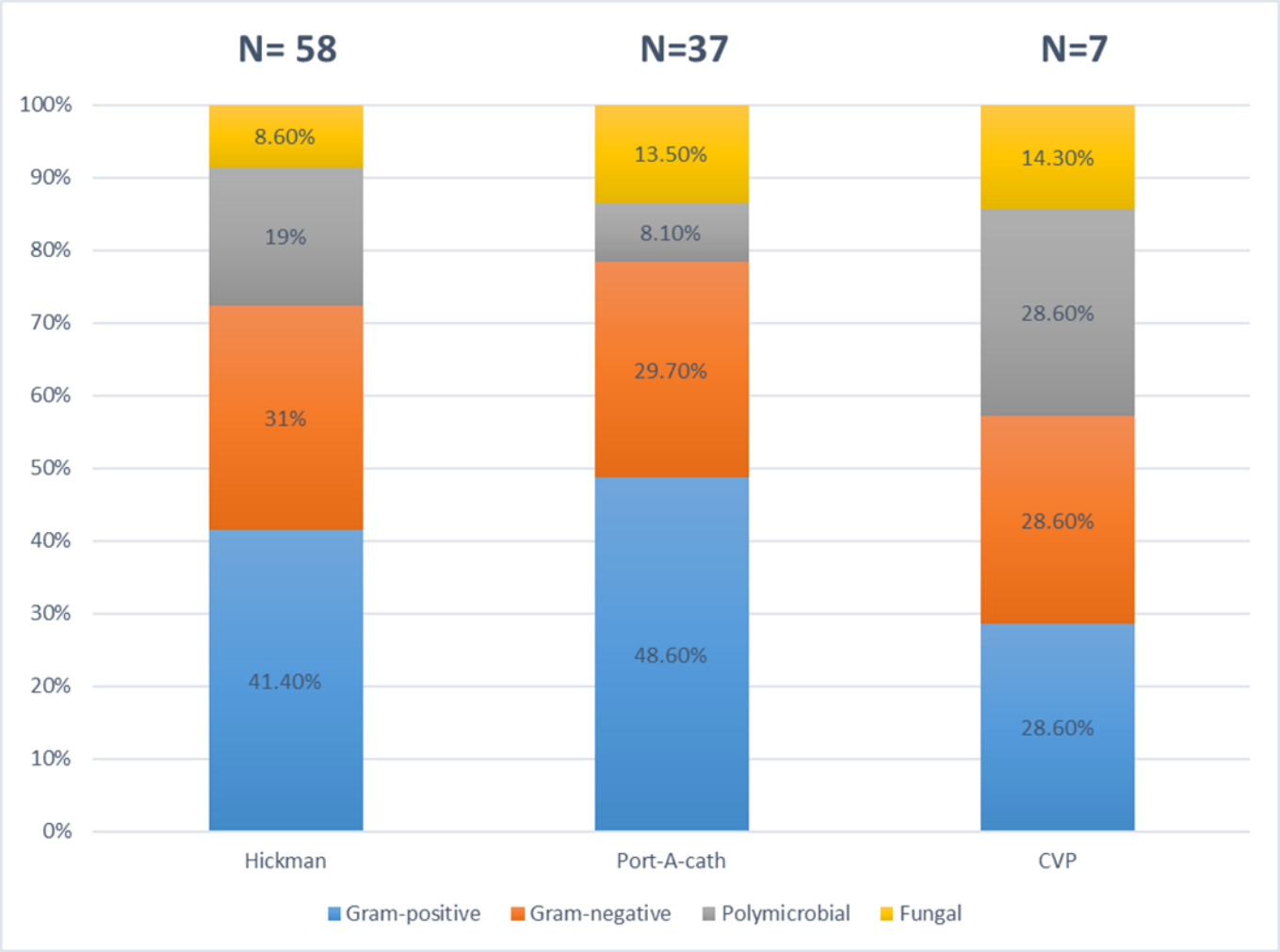
Pathogen	AML n (%) N=59	ALL n (%) N=47	NHL n (%) N=22	Solid cancers n (%) N=41	Brain cancers n (%) N=4	All blood culture isolates N=173	Proportion (%)
Gram-positive bacteria	**30 (50.8)**	**25 (53.2)**	**13 (59.1)**	**17 (41.5)**	**0**	**85**	**49.1**
Coagulase negative staphylococci	11 (18.6)	15 (31.9)	9 (40.9)	5 (12.2)		40	23.1
Viridans Group Streptococcus	16 (27.1)	1 (2.1)	1 (4.5)	5 (12.2)		23	13.3
Enterococcus species	3 (5.1)	2 (4.3)	3 (13.6)	1 (2.4)		9	5.2
Streptococcus pneumoniae		3 (6.4)		3 (7.3)		6	3.5
Staphylococcus aureus		4 (8.5)		1 (2.4)		5	2.9
Bacillus species				2 (4.9)		2	1.2
Gram-negative bacteria	**24 (40.7)**	**15 (31.9)**	**8 (36.4)**	**21 (51.2)**	**4 (100)**	**72**	**41.6**
Escherichia coli	9 (15.3)	7 (14.9)	1 (4.5)	1 (2.4)	1 (25)	19	11.0
Klebsiella species	9 (15.3)	2 (4.3)	1 (4.5)	4 (2.3)	2 (50)	18	10.4
Enterobacter species		1 (2.1)	1 (4.5)	5 (12.2)		7	4.0
Serratia marcescens	1 (1.7)		2 (9.1)			3	1.7
Salmonella species				2 (4.9)	1 (25)	3	1.7
Proteus mirabilis			1 (4.5)			1	0.6
Acinetobacter species	2 (3.4)	1 (2.1)	1 (4.5)	3 (7.3)		7	4.0
Pseudomonas aeruginosa	1 (1.7)	2 (4.3)	1 (4.5)	2 (4.9)		6	3.5
Stenotrophomonas maltophilia	2 (3.4)	1 (2.1)		1 (2.4)		4	2.3
Haemophilus influenzae				3 (7.3)		3	1.7
Moraxella species		1 (2.1)				1	0.6
Fungi	**5 (8.5)**	**7 (15)**	**1 (4.5)**	**3 (7.3)**	**0**	**16**	**9.3**
Candida parapsilosis	2 (3.4)	2 (4.3)	1 (4.5)			5	3
Candida albicans	1 (1.7)	1 (2.1)		2 (4.9)		4	2.3
Candida krusei	2 (3.4)	2 (4.3)				4	2.3
Candida tropicalis		2 (4.3)		1 (2.4)		3	1.7

Table 4: Antimicrobial susceptibility results

Antibiotic	Number of isolates tested	Number (%) of isolates resistant
I. Gram-positive bacteria (n= 85)		
Rifampicin	23	21 (91.3)
TMP/SMX	38	32 (84.2)
Cloxacillin	38	32 (84.2
Ampicillin	74	61 (82.4)
Ciprofloxacin	7	5 (71.4)
Erythromycin	43	29 (67.4)
Clindamycin	37	21 (56.8)
Vancomycin	54	3 (5.6)
Linezolid	27	1 (3.7)
II. Gram-negative bacteria (n= 72)		
Ampicillin	42	34 (81)
TMP/SMX	64	46 (71.9)
Co-Amoxiclav	41	27 (65.9)
Tobramycin	8	4 (50)
Ceftriaxone	42	21 (50)
Ceftazidime	55	26 (47.3)
Cefepime	58	24 (41.4)
Nalidixic acid	12	4 (33.3)
Gentamicin	44	12 (27.3)
Piperacillin - tazobactam	34	9 (26.5)
Amikacin	58	14 (24.1)
Ciprofloxacin	63	15 (23.8)
Colistin	40	7 (17.5)
Meropenem	56	3 (5.4)
Imipenem	56	3 (5.4)
Ertapenem	45	1 (2.2)
Fungal isolates (n= 16)		
Fluconazole	16	5 (31.3)
5-Fluorocytosine	14	3 (21.4)
Amphotericin B	15	0 (0)

Abbreviations: TMP/SMX, Trimethoprim-sulfamethoxazole.

Figure 1: Type of infectious agent per VAD type.



3 Appendices

3.1 Data Collection Sheet

Study Title: Epidemiology of Blood stream infections in Oncology patients at Red Cross War Memorial Children`s Hospital, Cape Town:

Biographical data:

Study number		
Folder number		
Date of Blood culture		
Date of Birth :		Age at time of blood culture
Gender	Male	Female
Primary Oncological diagnosis		
Date of Diagnosis		
Staging of Oncological diagnosis		
Additional underlying diagnosis if any		
Weight (Kg)	Weight for Age (Z-score)	

Treatment:

Chemotherapy	Yes	No
If yes, phase of treatment : (chemotherapy block)		
Chemotherapeutic medications within the last 21 days to blood culture	Name	Route

Medication 1		
Medication 2		
Medication 3		
Medication 4		
Medication 5		
Medication 6		
Medication 7		
Medication 8		
Date of starting chemotherapy block		
Date of last chemotherapy dose prior to blood culture		
Date of ending block		
Number days between last day of chemotherapy to blood culture		
Radiotherapy	Yes	No
Site/ Field of Radiotherapy		
Radiotherapy dose		
Date Radiotherapy commenced		

Date Radiotherapy completed		
Surgery in the last 3 months	Yes	No
If Yes, date of surgical procedure		
Surgical procedure(s)		
Haematopoietic Stem Cell Transplantation (HSCT)	Yes	No
If Yes, Day post HSCT		
Biological agents given?	Yes	No
If yes what agents and date	Agent 1	Date
	Agent 2	Date
Was the patient undergoing relapse of the malignancy?	Yes	No
If yes, number of relapse		

Haematological Investigations at time or closest to blood culture:

Full Blood Count (FBC) at time of blood culture	**Date:**	
White Cell Count		$x10^9/L$
Neutrophil Count	ANC $x10^9/L$	Proportion%
Monocyte Count	AMC $x10^9/L$	Proportion%
Lymphocyte Count	ALC $x10^9/L$	Proportion%
Haemoglobin		g/dL

Platelet count	$x10^9/L$
Date of onset of neutropenia:	
Date of last bone marrow aspiration and trephine biopsy:	
From bone marrow findings was patient in remission?	
Metastasis of tumour to other organs or systems at time of infection? Yes No	
If yes which organs/ systems involved:	

HIV infection

Patient tested for HIV			YES	NO		UNKNOWN	
HIV Test	Date			Result			
Rapid							
HIV ELISA							
HIV DNA PCR							
CD4 completed	YES	NO		UNKNOWN			
CD4 date							
CD4 absolute count		CD4 percentage					
Most recent Viral load at time of blood culture		Date		Log			

VAD

Presence of Vascular access device? (circle)	**Yes**	**No**
If yes, type of VAD		

(circle)	**Port-A-cath**	**Hickman line**
	Broviac line	**Other central line (specify)**
Date of insertion of VAD.		
Any abnormal symptoms or signs of from site of VAD	**Yes**	**No**
If yes	**Symptom**	**Sign**

Occurrence of fever

Fever:	Temperature at time of blood culture sample collection °C

Focus of infection

Pneumonia	Urinary tract infection
Meningitis	Soft tissue infection
Septic arthritis	Osteomyelitis
Catheter related blood stream infection	Other (specify)

Blood culture

Date of collection				
Cultured organism(s)				
Time from inoculation to growth (hours)				
Site of blood sample collection	**Peripheral site**		**VAD**	
Antibiotic sensitivity to cultured organism				
Piptazobactam	**S**	**I**	**R**	**N/S**
Amikacin	**S**	**I**	**R**	**N/S**
Ceftriaxone/ Cefotaxime	**S**	**I**	**R**	**N/S**
Ceftazidime	**S**	**I**	**R**	**N/S**
Cefepime	**S**	**I**	**R**	**N/S**
Ampicillin	**S**	**I**	**R**	**N/S**
Cloxacillin	**S**	**I**	**R**	**N/S**
Cotrimoxazole	**S**	**I**	**R**	**N/S**
Co Amoxiclav	**S**	**I**	**R**	**N/S**
Erythromycin	**S**	**I**	**R**	**N/S**
Ciprofloxacin	**S**	**I**	**R**	**N/S**
Clindamycin	**S**	**I**	**R**	**N/S**
Imipenem	**S**	**I**	**R**	**N/S**
Ertapenem	**S**	**I**	**R**	**N/S**
Meropenem	**S**	**I**	**R**	**N/S**
Vancomycin	**S**	**I**	**R**	**N/S**
Colistin	**S**	**I**	**R**	**N/S**

Linezolid	**S**	**I**	**R**	**N/S**
Rifampicin	**S**	**I**	**R**	**N/S**
Tobramycin	**S**	**I**	**R**	**N/S**
Nalidixic acid	**S**	**I**	**R**	**N/S**
Fluconazole	**S**	**I**	**R**	**N/S**
Amphotericin B	**S**	**I**	**R**	**N/S**
Foriconazole	**S**	**I**	**R**	**N/S**
Other (specify)	**S**	**I**	**R**	**N/S**

Prior antibiotics.

Any antibiotic commenced within 5 days prior to positive blood culture?	

Name	**Date Commenced**	**Date stopped**	**Route**	**Duration**

Antibiotic(s) commenced at time of blood culture sample collection (empiric antibiotics):

Name	**Date Commenced**	**Date stopped**	**Route**	**Duration**

Use of alternative line antibiotics/ antifungals

Second line antibiotics / antifungals commenced?			**Yes**	**No**
If Yes				
Name	**Date Commenced**	**Date stopped**	**Route**	**Duration**
Time to effective antibiotic therapy: (Duration between blood culture collection to commencement of antibiotics organism cultured is sensitive to) (days)				

Complications:

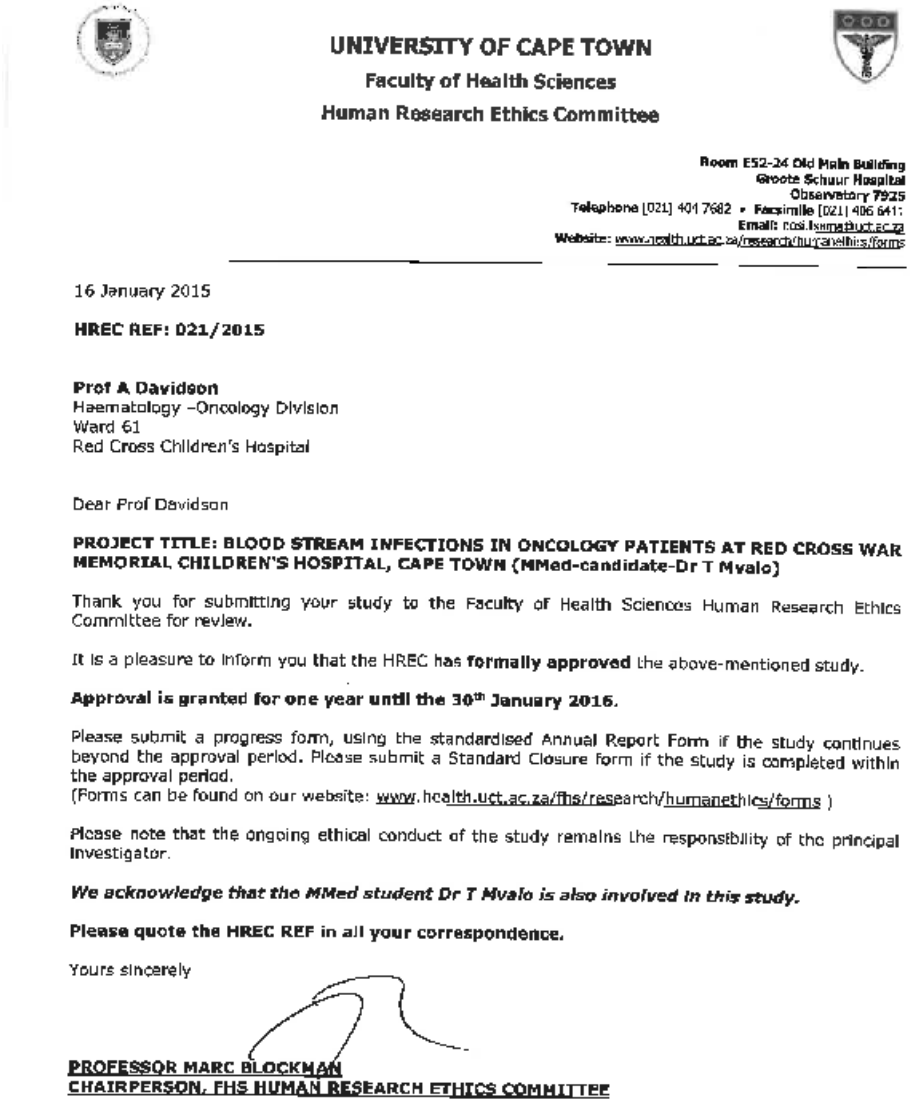
Septic shock	Respiratory failure
Coma	Disseminated Intravascular coagulation
Renal failure	Liver failure
Other (specify)	
Maximal level of care provided Need for ICU admission	
Oncology ward (G1) care	

Any limitation of care to patient (Intervention level), if Yes Specify

Hospital outcome from infection

Discharged	**Death**
Date of discharge / death	
If outcome death, cause of death:	
Post-mortem done after death? **If yes, post-mortem findings**	

3.2 Ethics approval



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 641:
Email: rosi.lyons@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

16 January 2015

HREC REF: 021/2015

Prof A Davidson
Haematology –Oncology Division
Ward 61
Red Cross Children's Hospital

Dear Prof Davidson

PROJECT TITLE: BLOOD STREAM INFECTIONS IN ONCOLOGY PATIENTS AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN (MMed-candidate-Dr T Mvalo)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th January 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

We acknowledge that the MMed student Dr T Mvalo is also involved in this study.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

3.3 Instructions to Authors of the target journal

The Pediatric Infectious Disease Journal (PIDJ)
Online Submission and Review System

Author Resources

Instructions for Authors (this page)
Reprint Ordering
Permissions Requests

SCOPE
The Pediatric Infectious Disease Journal is a peer-reviewed, multidisciplinary journal directed to physicians and other health care professionals who manage infectious diseases of childhood.

New Policy, effective for all articles submitted on or after April 15, 2017

Articles that have received funding by major pharmaceutical companies, except Letters to the Editor, will be required to pay the following publication charges. The universal fee for all accepted manuscripts with major pharma funding is: $2000.00 US, plus an additional per-page fee with 2 options: 1) $50 per page for print and online publication; or 2) $25 per page for online only publication. Once published, these articles will be available online by free access.

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Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker’s bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

In addition, each author must complete and submit the journal's copyright transfer agreement, which includes a section on the disclosure of potential conflicts of interest based on the recommendations of the International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (www.icmje.org/update.html).

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PREPARATION OF MANUSCRIPT

Manuscripts that do not adhere to the following instructions are returned to the corresponding author for technical revision before undergoing peer review. Also, to streamline the review process, on reviewing newly submitted manuscripts, we will identify those that do not meet the mission of the journal, provide no new information or insights into management of infectious diseases or are of more local importance and better suited for a regional journal and return them immediately to the authors to allow them to submit their work elsewhere in a timely fashion. Case series take preference over single case reports.

New Article Types

Research Reports This section comprises manuscripts on all aspects of the molecular pathogenesis and immunologic mechanisms of bacterial, viral, fungal and other infections in infants, children and adolescents. The emphasis will be on manuscripts that present data that

are clinically applicable and provide a more thorough understanding of the pathophysiologic basis of infections in children and that could impact eventual treatment and prevention. The manuscripts can be formatted as original studies or brief reports and will be peer reviewed.

HIV Reports The section comprises of high-quality, high-impact original articles and brief reports of epidemiologic, clinical, translational and implementation science studies pertaining to the prevention, treatment and outcomes of HIV infection in infants, children, and adolescents.

New Policy, effective for all articles submitted on or after April 15, 2017
Articles that have received funding by major pharmaceutical companies, except Letters to the Editor, will be required to pay the following publication charges. The universal fee for all accepted manuscripts with major pharma funding is: $2000.00 US, plus an additional per-page fee with 2 options: 1) $50 per page for print and online publication; or 2) $25 per page for online only publication. Once published, these articles will be available online by free access.

Vaccine Reports Articles that present data from Vaccine Phase II-IV studies will appear in this section. These manuscripts receive the same peer review as articles submitted as Original Studies. The universal free access fee for all accepted manuscripts in this category is: $2000.00 US, plus an additional per-page fee with 2 options: 1) $50 per page for print and online publication; or 2) $25 per page for online only publication. All articles in this series will be available online by free access. For manuscripts in this category, authors should refer to the "Guidelines for collection, analysis and presentation of vaccine safety data in pre- and post-licensure clinical studies" published in Vaccine (2009, vol. 27; pp 2282-8) and use case definitions as developed by The Brighton Collaboration (www.brightoncollaboration.org) whenever possible.

ESPID Reviews and Reports (or purple pages) This section comprises invited concise reviews on all aspects of infections in infants, children and adolescents including bacterial, viral, fungal and parasitic infections. Reviews on prevention, diagnosis, therapeutic interventions and drugs as well as on teaching and conferences in pediatric infectious diseases are the focus of this section and will concentrate on novel findings, development and controversial issues.

Manuscript Submission

Online manuscript submission: All manuscripts must be submitted on-line through the new web site at http://pidj.edmgr.com/. First-time users: Please click the Register button from the menu above and enter the requested information. On successful registration, you will be sent an E-mail indicating your user name and password. Print a copy of this information for future reference. Note: If you have received an E-mail from us with an assigned user ID and password, or if you are a repeat user, do not register again. Just log in. Once you have an assigned ID and password, you do not have to re-register, even if your status changes (that is, author, reviewer, or editor). If you experience any problems, please contact Amy Newman, Journal Manager, at PIDJournal@outlook.com, Ph 830-865-1249, Fax 214-710-2175.

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Title page: **Title page must be submitted as a separate file**. Include on the title page: (a) complete manuscript title; (b) authors' full names, highest academic degrees, and affiliations; (c) name and address for correspondence, including Fax number, telephone number, and E-mail address; (d) all sources of support, including pharmaceutical and industry support, that require acknowledgment; (e) list three to five key words for indexing; (f) an abbreviated title of 55 characters or less used for the cover of the journal; and (g) a running head title of 44 characters or less including spaces used for page headings on the pages in which your article is published.

The title page must also include disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

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Letters to the Editors: Letters to the Editors should pertain to articles published within the Pediatric Infectious Disease Journal or highlight important new clinical or laboratory insights. Text should contain 500 words or fewer and less than 5 references.

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Text: Organize the manuscript into four main headings, Introduction, Materials and Methods, Results, and Discussion. If a brand name is cited, supply the manufacturer's name and address (city and state/country).

Abbreviations: For a list of standard abbreviations, consult the *American Medical Association Manual of Style*, 9th edition, or other standard sources. Write out the full term for each abbreviation at its first use unless it is a standard unit of measure. Abbreviations are allowed only if used three times or more in text.

References: The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. Cite the references in text in the order of appearance, including those references cited in tables and figure legends at the chronological citation of the tables and figures in text. Cite unpublished data, such as papers submitted but not yet accepted for publication or personal communications, in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names, or access the list at **http://www.nlm.nih.gov/tsd/ serials/lji.html**. Sample references are given below.

Journal article
1. Trujillo M, Correa N, Olsen K, et al. Cefprozil concentrations in middle ear fluid. *Pediatr Infect Dis J*. 2000;19:268—270.

Book chapter
2. Grose C. Bacterial myositis and pyomyositis. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 1998:704—708.

Entire book
3. Nelson JD, Bradley JS. *Nelson's Pocket Book of Pediatric Antimicrobial Therapy*. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.

Proceedings
4. Harrigan PR, Dong W, Weber AE, et al. Highly mutated RT and protease [Abstract I-115]. In: 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, September 24 to 27, 1998. Washington, DC: American Society for Microbiology; 1998.

Online journals
5. Friedman SA. Preeclampsia. Obstet Gynecol. [serial online]. January 1988;71:22–37.

World Wide Web
6. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS web site]. June 1, 1996. Available at: http://www.ama-assn.org/special/hiv/ethics. Accessed June 26, 1997.

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A) Creating Digital Artwork

1. Learn about the publication requirements for Digital Artwork: http://links.lww.com/ES/A42
2. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

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Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

Remember:

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- Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.

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We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive (see Table, Supplemental Digital Content 2).

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