Klebsiella pneumoniae bloodstream infections in hospitalised children at Red Cross War Memorial Children's Hospital: 2006-2011

by

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BYSHEL001

A research report submitted to the Faculty of Health Sciences, University of Cape Town, South Africa, in fulfilment of the requirements for the Master of Science

(Paediatric Medicine) degree

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of submission: 14th August 2015

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DECLARATION

I, Heloise Buys, declare that this dissertation is my own work (except where acknowledgements indicate otherwise). It is being submitted for the Master of Science (Paediatric Medicine) degree at the University of Cape Town and that no part of it has been submitted for another degree at any university.

Signed by candidate

Signature: signature removed

Date: 14th August 2015

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

ORAL PRESENTATIONS

- Buys H, Bamford C, Kranzer K, Madikane E, Whitelaw A, Reichmuth K, and Eley B. Bacteraemic *Klebsiella pneumoniae* infections in hospitalised children at Red Cross War Memorial Children's Hospital over a 4-year period 2007-2010 (An interim analysis). University of Cape Town School of Child and Adolescent Health Annual Research Day, 11th October 2011, Cape Town.

- Buys H, Muloiwa R, Bamford C, and Eley B. *Klebsiella pneumoniae* bloodstream infections in hospitalised children at Red Cross Children's Hospital 2006-2011. Department of Paediatrics and Child Health academic meeting, Red Cross War Memorial Children's Hospital, Cape Town, 1st August 2012.

ABSTRACT

Background:

Klebsiella pneumoniae (KP) is a significant paediatric bloodstream pathogen in children. There is little data about KP in South African children. The focus for the present study was to address this knowledge gap.

Methods: This study addressed a retrospective case notes review on bloodstream *Klebsiella pneumoniae* infections at a children's hospital in Cape Town, South Africa 2006-2011 using conventional descriptive and comparative statistical methods.

Results: Of 410 hospitalised children with laboratory confirmed KP bloodstream infection (KPBSI), 339 (83%) were caused by presumed extended-spectrum β-lactamase (ESBL) producing isolates. The median age (interquartile range, IQR) was 5.0 (2-16) months, 212 (51.7%) were male, 82 (20%) were HIV-infected, and 241 (58.8%) were moderately or severely underweight. The infection was nosocomial or healthcare-associated in 389 (95%) children and community-acquired in 21 (5%) children. Significant risk factors for the acquisition of ESBL-KP bloodstream infection included cephalosporin exposure in the preceding 12 months prior to the KPBSI p=<0.0001: aRR 1.27 (95% CI: 1.15-1.40); HIV infection p=0.01: aRR 1.12 (1.02-1.22), and those who had intravenous infusions for more than 3 days prior to the KPBSI, p=0.002: aRR 1.21(95% CI: 1.07-1.36).

The median age of 123 (30%) children who died was four (IQR 1-15) months. The median, IQR, time between KPBSI and death was four (IQR 1-13) days. Patients with significant risk factors for death included those having HIV-infection, p=<0.0001: aRR 1.8(95% CI: 1.32-2.45); having excoriated skin, p=<0.0001: aRR 1.74 (95% CI: 1.31-2.32); being in PICU, p= 0.01: aRR 1.61 (95% CI: 1.13-2.30) or needing PICU admission, p= 0.004: aRR 1.7 (95% CI: 1.18-2.45).

Conclusion: ESBL-producing KP is an important cause of laboratory confirmed bloodstream infection at this children's hospital and is associated with high mortality. Improved infection control practice and antibiotic stewardship are essential for controlling this pathogen.

Key words: *Klebsiella pneumoniae* bloodstream infection, children, Africa

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ACKNOWLEDGMENTS

I would like to thank…

My supervisor Professor Brian Eley for stimulating this discussion and journey. His outstanding knowledge and extensive research experience have enriched my study programme greatly. His encouragement when achievement and success seemed unfeasible has been priceless; his support and lessons in attention to detail will not be forgotten- those dreaded moments of manuscript review knowing there would be many more hours of comments and suggestions to work through! Thank you it was worth it!

Dr Colleen Bamford who patiently answered my numerous untimely questions and supported me throughout the journey, offered advice and support on laboratory and microbiological technicalities; thank you too for facilitating a visit to the laboratory giving me insight into the processes involved in the processing of specimens and laboratory innuendo. Thanks to the National Health Laboratory Service for readily providing the years of laboratory data.

My colleagues Ralph Diedericks and Rudzani Muloiwa who provided moral support and encouragement- thank you to you both. Special thanks to Rudzani for coaching and patiently supporting me throughout the statistical analysis; you've empowered me with a valuable skill enabling me to analyse scientific data- strengthening any further research I may undertake in the future. Your words of constant encouragement and support have sustained my efforts throughout this preparation period and will remind me of the task ahead.

Red Cross War Memorial Children's Hospital Records Department for helping with folder retrieval despite the numerous demands upon them.

Naiema Salie and Tessa Strauss for providing the hospital admission and death statistics so willingly.

Sr. Spasina King for helping with the data capturing- trawling through case files is never an easy job!

Dr Komola Pillay and the Department of Histopathology at RCWMCH for access to the post mortem results of 15 children who died at the hospital as a result of KP BSI.

Ilhaam Farhquar and Ayesha Apolles who enthusiastically and tirelessly retrieved folders for the review and remained ever cheerful and keen to learn.

Thank you Dr Kirsten Reichmuth for helping with the design of a complex database. Thanks to Dr Katherina Kranzer and Dr Vash Pillay for giving sound advice and assistance with some of the data analysis.

The Harry Crossley Foundation generously funded a 4- month sabbatical during which the foundations of this research project were laid.

DEDICATIONS

I would like to dedicate this thesis to my sons Ben and David who wonder why so much learning continues after school is done - I love you!

My dearest Mother Dolly and siblings Margaret, James, Ursula, Bernadette, Averille and Linda who were there all the way in spirit- hey guys, this is for us! The memory of my Dad's tenacity, resilience and unshakeable sense of Ubuntu guided my perseverance and reminds me that improving the lot of those vulnerable or less fortunate offers lasting reward.

To the children who come to Red Cross War Memorial Children's Hospital and suffer numerous setbacks on the road to recovery- may this work help inspire your clinicians into a better way; for those that did not make it- rest in peace.

"... and now this work is ended, be Thou its blessing and completion..."

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GLOSSARY OF ABBREVIATIONS

This document contains the following abbreviations:

ALT	alanine transaminase
AST	aspartate transaminase
aOR	adjusted odds ratio
APTT	activated partial thromboplastin time
aRR	adjusted risk ratio
ART	antiretroviral therapy
ATN	acute tubular necrosis
BP	blood pressure
BSI	bloodstream infection
CA	community acquired
CAI	community acquired infection
CDC	Centers for Disease Control and Prevention
CRF	case record file
CRP	C-reactive protein
DIC	disseminated intravascular coagulopathy
ESBL	extended-spectrum β-lactamase
ESBL KP	presumed extended-spectrum β-lactamase producing *Klebsiella pneumoniae*
eGFR	estimated glomerular filtration rate
FBC	full blood count
HAI	healthcare-associated infection
HRF	healthcare-associated risk factors
HIV	human immunodeficiency virus
KP	*Klebsiella pneumoniae*
KP BSI	*Klebsiella pneumoniae* bloodstream infection
IMCI	Integrated Management of Childhood Illness
IQR	interquartile range
LCBSI	laboratory confirmed bloodstream infection
LOS	length of stay
MODS	multiorgan dysfunction
MSSA	methicillin sensitive *Staphylococcus aureus*

NPA	nasopharyngeal aspirate
NEC	necrotising enterocolitis
NHLS	National Health Laboratory Service
OR	odds ratio
PJP	*Pneumocystis jirovecii* pneumonitis
PCT	procalcitonin
PICU	Paediatric intensive care unit
PIDU	Paediatric infectious diseases unit
PT	prothrombin time
RCWMCH	Red Cross War Memorial Children's Hospital
RR	risk ratio
SD	standard deviation
SSW	short stay ward
USA	United States of America
UTI	urinary tract infection
UWFA	underweight-for-age
WAZ	weight-for-age z-score
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 The problem of *Klebsiella pneumoniae*:

Klebsiella pneumoniae (KP) are Gram-negative bacteria from the *Enterobacteriaceae* family. This large family includes the genera *Citrobacter, Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella*, and *Yersinia*. These bacteria are all facultative anaerobes that are oxidase negative; they also share the ability to ferment glucose, reduce nitrates to nitrites, and most are motile.

Klebsiella species are found in two main places: in natural environmental habitats like soil, surface water collections, and sewage and on mammalian mucosal surfaces. Three major species of *Klebsiella* are of clinical relevance: *KP, oxytoca,* and *rhinoscleromatis. KP* are by far the most commonly isolated species and the most clinically important; they colonise mainly the upper respiratory tract and extensively inhabit the colon. They have tenacious mucoid polysaccharide capsules that protect them against phagocytosis and antibiotic destruction while contributing to their cytotoxic effect. Early literature often used the name *Bacillus mucosus capsulitis,* somewhat depicting these properties (Bensley, 1932). *KP* belong to the group of biofilm-associated organisms. Biofilm formation has been described in patients with any foreign indwelling medical devices. Indwelling vascular catheters, endotracheal tubes, and urinary catheters are particularly associated with *KP* biofilm formation. Biofilm provides protection for the adhering microorganisms against the host's immune system, antibiotic penetration, and a medium in which to exchange resistance plasmids (Donian, 2001); as such the biofilm nidus provides an ever-present risk for invasive nosocomial infections. Studies have shown the presence of

fimbriae in all *KP* isolates to mediate adherence to indwelling device surfaces (Schroll et al., 2010). Unlike the other *Enterobacteriaceae,* they are non-motile.

Under antibiotic selection pressure, KP acquire and produce a variety of beta (β)-lactamase enzymes that confer antibiotic resistance mainly by hydrolysing the β-lactam rings of antibiotics. Extended-spectrum beta-lactamases (ESBLs) are a particular type of plasmid-encoded beta-lactamase enzyme. This enzyme is able to hydrolyse a wide spectrum of β-lactam antibiotics including 3rd and 4th generation cephalosporins.

ESBL genes may be located on bacterial chromosomes but are primarily located on plasmids through which they can be transferred between Gram-negative bacteria by translocation. Examples of extended-spectrum beta-lactamases include TEM, SHV, and CTX-M each of which is associated with sub-variants differing in expression only by regional location. Researchers have described over 1000 variants (Malloy and Campos, 2011). Any given organism may express more than one type of ESBL. These multidrug resistant organisms may disseminate within and between wards and hospitals, ultimately becoming a global problem.

Findings of patients and retail poultry meat with the same ESBL plasmids, genes, and strains provided some evidence of horizontal transmission from animals to humans. This horizontal transmission is most likely via the food chain. Researchers thought there was a link between this phenomenon and high antibiotic use in the poultry industry (Mesa et al., 2006). Other areas of animal husbandry also make extensive use of antibiotics. Antibiotic selection pressure for resistance and plasmid dissemination drives the spread of resistance.

Colonised patients serve as an infection reservoir in humans. In one study, researchers showed that up to 75% of patients remain colonised with ESBL-producing organisms for at least 2 months and that colonisation lasted for more than 1 year in up to 25% of patients (Leverstein-van Hall and Muilwijk, 2010). In another study, the median duration of faecal carriage in 51 infants discharged from a neonatal intensive care unit was 12.5 (IQR 9-17.5) months, with the longest time of 23.5 months in one infant. Furthermore, it appeared that the colonised infants transmitted the CTX-M-15 *KP* isolate to 20% of other household members. Thus, this magnified the serious concerns on the ease of dissemination and colonisation by resistant KP (Löhr et al., 2013).

KP bacteria may cause serious multi-organ disease that is associated with high mortality. The spectrum of clinical infectious manifestations caused by *KP* bacteria include:

- pneumonia
- urinary tract infections (UTI)
- biliary tract infection
- wound and soft tissue infection
- abscess formation
- bone infection
- meningitis
- diarrhoeal disease
- necrotising enterocolitis (NEC)
- bloodstream infections (BSI)

Early signs of a BSI, whether nosocomial or community acquired, include fever, tachycardia, tachypnoea, and general malaise. In neonates, early signs include apnoea, temperature instability, skin mottling, convulsions, drowsiness, irritability, poor feeding, and feed intolerance may be other more subtle manifestations (WHO, 2005). Uncontrolled sepsis can escalate to septic shock and multi-organ failure in a short period.

1.2 Literature search

A MEDLINE/ Pubmed database search was conducted from 1966 to December 2011 for English language studies of humans for articles that reported KP BSI in children from birth to 18years of age. The search terms included:

- (child or children or infant or adolescent) AND
- (paediatrics or paediatrics; paediatric or pediatric) AND
- (sepsis or septicaemia or septicaemia) AND
- (bacteraemia or bacteremia or blood circulation or bloodstream or bloodstream infection or bloodstream infections) AND
- (*Klebsiella* or *Klebsiella pneumoniae*) AND
- (extended spectrum beta lactamase or ESBL) AND
- (risk factors)

Four hundred and eighty five titles and abstracts of citations identified from the literature search were screened for relevance on paediatric KP bloodstream infections; case reports with <20 subjects, study groups including adults, children with mixed infections, studies exclusively involving neonates and preterm infants were excluded. Nineteen studies were deemed relevant. Additional searches were done on the reference sections of relevant studies.

1.3 *Klebsiella pneumoniae* bloodstream infections – the global picture

One of the inevitable consequences of antibiotic use has been the microorganisms' survival strategy through varying degrees of antimicrobial resistance (Datta and Hughs, 1983). Moreover, beta-lactamases were evident even before antibiotics were introduced into clinical use. The first documentation of plasmid transfer of cephalosporin antimicrobial resistance from nosocomial *KP* strains to *E Coli* was in Germany in 1983, over 30 years ago. In laboratory-based experiments, researchers were able to demonstrate conclusively the transfer of resistance plasmids between Gram-negative species from isolates taken from patients attending an outpatient clinic. Three serologically different clones of *KP*, resistant to cefotaxime, cefuroxime, and gentamicin but sensitive to cefoxitin, were readily transmissible to *E Coli* recipient strains after cultivating the carefully mixed incubated broth donor and recipient cultures and then re-evaluating the strains and their sensitivity patterns (Knothe et al., 1983).

Thus, it appeared that the introduction of the powerful 3rd-generation cephalosporins into clinical use in 1981, in the US and South Africa, played a fundamental role in the evolution of the extended-spectrum beta-lactamases (ESBLs). Soon after, many more reports emerged, initially in the adult literature. It soon became clear that hospitalised children across the globe have not been immune to this problem (Aiken et al., 2011; Al-Zamil, 2008; Arnoni et al., 2007; Blaschke et al., 2009; Blomberg et al., 2005; Jaspan et al., 2008; Kim et al., 2002; Marra et al., 2006; Moyo et al., 2010; Pérez-González et al., 2007; Qin et al., 2008; Raymond et al., 2007; Singhi et al., 2008; Woerther et al., 2011; Zaoutis et al., 2005).

As more antibiotics were introduced into the armamentarium against bacteria, so too did ß-lactamase combinations become more complex and diverse. Researchers first reported plasmid-mediated ESBL resistance to quinolone antibiotics conferring broad-spectrum resistance to hospital isolates of *KP* and *E Coli* in 1998. This provided evidence of the spread of quinolone-resistant mutants between *Enterobacteriaceae* (Martínez-Martínez at al., 1998).

Not only is the problem a global one but a new threat has emerged with the carbapenemase-producing KP. This is a bacterial response to the more liberal use of carbapenem antibiotics as well as the continued abuse of all major antibiotic classes in both adult and paediatric populations. This review does not include KP carbapenamase resistance mainly because it emerged as a clinical problem at Red Cross War Memorial Children's Hospital (RCWMCH) in 2012 and therefore falls outside the study period. However, researchers first reported KP carbapenamase resistance in South Africa in private hospitals in 2011, and it is likely to become a formidable clinical problem (see Table 1.1) (Brink et al., 2012).

Table 1.1: Key milestones in the development of antibiotic resistance in *Enterobacteriaceae*, with particular reference to *Klebsiella pneumoniae (KP)*

Date of first clinical use of selected antibiotics	Resistance documentation in Gram-negative bacteria	Reference
1928 Penicillin discovered		Fleming, 1929
1940 Penicillin	1940 Penicillinase (beta-lactamase)-producing *E Coli*	Abraham and Chain, 1940
	1963 TEM-1 beta-lactamases in *E Coli*	Datta and Kontomichalou, 1965
1981 3rd generation cephalosporins (Cefotaxime)	1983 ESBL- *KP* resistance plasmids	Knothe et al., 1983
1985 Carbapenems (Imipenem)	1994 KP Carbapenemase gene	Naas and Norsmann, 1994
1962 Quinolones	1998 KP Quinolone resistance	Martínez-Martínez et al., 1998

KP: *Klebsiella pneumoniae*
E Coli: Escherichia Coli
ESBL: extended-spectrum beta-lactamase
TEM-1: the first described and most widespread beta-lactamase (resistance only to penicillin and ampicillin)

A systematic review of 16 studies involving 215 bloodstream or lung isolates from children younger than 5 years with pneumonia in developing countries showed high risk of death in malnourished children with pneumonia. Researchers conducted ten of these studies in Sub-Saharan Africa and Asia. Within the studies, there were 1062/4487 moderately malnourished children and 1866/11 497 severely

malnourished children. Their relative risk for mortality ranged from 1.2 to 36.5 for moderately malnourished children and 2.9 to 121.2 for severely malnourished children with pneumonia in comparison to children without. In severely malnourished children, *KP* and *Staphylococcus aureus* (MSSA) were the top two bacterial isolates implicated in the aetiology of the pneumonia in 26% and 25% of 215 isolates from all studies that described the spectrum of pathogens. *Streptococcus pneumoniae (18%), Escherichia coli (8%), Haemophilus influenzae (8%),* and *Salmonella spp*. (5%) were the next most commonly isolated organisms. Researchers did not evaluate the contribution of these individual pathogens to mortality (Chisti et al., 2009).

In the U.K., recent voluntary laboratory surveillance data on bacteraemia of selected *Enterobacteriaceae* from England, Wales, and Northern Ireland show that KP was the most common *Klebsiella spp.* The rate of laboratory-confirmed *Klebsiella spp.* infections per 100 000 population had increased from 10.8 to 11.3 over 2008-2012, whilst infection rates due to the other reported *Enterobacteriaceae*, *Enterobacter spp*., *Serratia spp*. and *Citrobacter spp*., had declined slightly. Though numbers were small, *KP* BSI was higher in two groups: among infants and among the elderly. The annual proportion of all *Enterobacteriaceae* BSI reported in this laboratory surveillance due to *KP* had increased from 40.3 % (4 162/10 318) to 50.7% (5 105/10 066) over the 5-year period. Antibiotic susceptibility testing showed resistance to 3rd-generation cephalosporins remained steady at about 10%, likely ESBL-related, over the 5 years. Resistance to the carbapenems was not common, and researchers first observed this in 2009. This study did not link clinical data to the laboratory isolates; therefore, clinical information of patients from whom the enterobacteriaceae were isolated, was not described (Public Health England, 2013).

1.4 *Klebsiella pneumoniae* bloodstream infections in African children

Not much published information exists on the extent and effect of *KP* in paediatric BSIs in Africa. A summary of the few reports that exist follows:

- A meta-analysis of community acquired BSIs in African adults and children found that *KP* is in fact not a common community-acquired bloodstream pathogen. Gram-negative organisms caused 54% of all the paediatric BSI from 22 studies conducted between 1984 and 2006. *Enterobacteriaceae* caused 36.8% of blood culture isolates, with *Salmonella spp.* being the most common genus in 21.4% of cases followed by non-salmonella *Enterobacteriaceae* in 15.3%; *E Coli* and *KP spp.* caused 9.4% and 2.8% of cases, respectively. This analysis showed that Gram-positive infections caused 43.4% of all BSIs; other organisms caused the remaining 2.6% of BSI. Researchers tested 6% of the 43 130 study children for HIV infections. Of those tested, the prevalence of HIV infection was 18.5% (Reddy et al., 2010). There were no South African studies in the analysis. Non-typhoid salmonella (NTS) is a common cause of Gram-negative BSI in many African countries to the north of South Africa (Feasey et al., 2001).
- In a Kenyan study, *Klebsiella spp.* ranked 9th out of 10 causes of bacterial isolates from 1094 paediatric Kenyan patients with bacteraemia (Berkley et al., 2005).
- African reports are scarce with respect to antemortem BSIs. In a 7-year prospective study involving more than 26 000 Kenyan children, researchers described *KP* as the second most common Gram-negative organism that accounted for 20% of healthcare associated infections. The most common Gram-negative organism was *E coli* while *S aureus* the most common Gram-positive pathogen, they accounted for 21% each, of all health-care associated infections. In

this study, researchers identified malnutrition and receipt of a blood transfusion as risk factors for healthcare-associated BSIs (Aiken et al., 2011).

- Researchers described outbreak reports of KP septicaemia in neonatal and paediatric units throughout Africa including Nigeria and Tanzania, associating these with high mortality (Iregbu and Anwaal, 2007; Blomberg et al., 2005). This link with hospitalisation is an important pointer to the epidemiology of KP BSI in children.

1.5 South African experiences of *Klebsiella pneumoniae* bloodstream infections in children

One of the first paediatric studies documenting the role of KP in childhood infection in South Africa showed that KP was one of the dominant organisms causing healthcare associated BSIs in malnourished children in Johannesburg (Berkowitz, 1984). In this 12-month prospective study conducted in 1982 at Chris Hani Baragwanath Hospital, a public hospital, researchers analysed community- and hospital-acquired BSI in children admitted to the general paediatric wards. Of 5397 admissions to the general paediatric wards, there were 337 significant bacterial bloodstream isolates from 315 children. The mean age was 19 months, and 64 (20%) children had a chronic underlying medical condition.

Over the study period, *Streptococcus pneumoniae* and *Salmonella enteritidis* were the most common organisms associated with community acquired pneumonia and gastroenteritis respectively, the first peaking in winter and the second peaking in summer. Nine of 22 (40.9%) were *KP* isolates, 3/8 (37.5%) were *Pseudomonas aeruginosa* isolates, and 7/21 (33.3%) were *Staphylococcus aureus* isolates causing nosocomial BSI in the study population. Susceptibility testing showed that 71% of the KP and 98% of the *E Coli* isolates were sensitive to Gentamicin. Researchers did not

report further sensitivity data for these organisms. In the patients with bacteraemia, the overall case fatality rate (CFR) was 23%; the CFR associated with KP BSI was higher at 32%. At this time, researchers did not test the children for HIV infection (see Table 1.2).

Table 1.2: Ranking of the top 6 bloodstream pathogens by case fatality rate in hospitalised children (Berkowitz, 1984)

Organism	Case Fatality Rate
Pseudomonas auruginosa	63
Escherichia Coli	33
Klebsiella pneumoniae	32
Haemophilus influenzae	23
Streptococcus pneumoniae	22
Salmonella enteritidis	20

Later, in 1989, another group of researchers described KP as one of the most common causes of nosocomial lower respiratory tract infections in hospitalised South African children in a study evaluating bloodstream pathogens. During the study period 8524 children were admitted to the paediatric wards at Tygerberg Hospital, a large public hospital. Researchers documented 171 cases of bacteraemia in 165 patients: 77.2% (n=132) and 22.8% (n=39) were community and hospital-acquired respectively. The mean age of the children with BSI was 2.4 years. The most common community-acquired bloodstream pathogens were *Streptococcus pneumoniae* (33% of cases), *Staphylococcus aureus* (13.6%), and *Neisseria meningitidis* (11.4%). Common hospital-acquired pathogens were *Klebsiella spp*. (18%) followed by *Salmonella spp.*

(13.4%). These Gram-negative BSIs occurred more commonly in malnourished children and in children with acute respiratory infections (Cotton et al., 1992).

The role of HIV infection in South African paediatric BSI was unknown at that time though unlikely to be significant. The first documented cases of HIV infection in South Africa was in 1983 (Ras et al., 1983).

The introduction of 3rd generation cephalosporin cefotaxime into the country to treat bacterial meningitis in 1981 may have contributed to the evolution of resistant KP. Although, this is unlikely to be the only explanation. Exposure to any antibiotic may induce bacterial resistance by imposing selective pressure.

In 1990, a Cape Town-based, 4-year survey found that children hospitalised with measles were six times more likely to develop a nosocomial BSI compared with other children in the general paediatric wards (OR 6.1; 95% CI 3.2-11.8). KP and *Salmonella* were the most likely Gram-negative organisms in 47/64 (73.4%) Gram-negative isolates, and 22 (29.7%) were due to KP. Researchers postulated that measles-induced immunosuppression was a major predisposing factor. The antibiotic resistance patterns at that time showed that although all the *KP* isolates were sensitive to cefotaxime and amikacin, 14/22 isolates were resistant to Gentamicin and chloramphenicol (Hussey and Simpson, 1990).

A public sector South African surveillance study was completed on susceptibility testing on bloodstream pathogens from mixed adult and paediatric patients using hospital and community acquired infections in 2010. These researchers drew isolates from a number of participating public sector laboratories in Johannesburg, Cape Town, Pretoria, and Bloemfontein. The data documented high levels of antibiotic

resistance among KP isolates. In particular, between 55 and 74% of the *KP* isolates were classified as ESBL- producers (see Table 1.3) (Bamford et al., 2011).

Table 1.3: Susceptibility testing for *Klebsiella pneumoniae* from the 2010 Antimicrobial susceptibility Surveillance Data (Bamford et al):

Antibiotic	Proportion sensitive
Gentamicin	30-51%
Ciprofloxacin	49-79%
Amikacin	66-98%
Ertapenem	96-98%
Meropenem/Imipenem	98-100%
Cefepime[#]	26-45%

[#] extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* inferred if the isolate was resistant to cefepime

South African National surveillance data over 2 years showed increasing reports of resistance to carbapenem antibiotics from different public institutions (see Table 1.4) (Perovic et al., 2013). Although the percentage changes were small, the absolute numbers showed an increase from 2012 to 2013.

Table 1.4: Comparison of selected antibiotic susceptibility profiles of KP from BSI at 13 South African public sector sites 2012 and 2013 (Perovic et al., 2013)

	2012				2013			
	2627 *Klebsiella pneumoniae* isolates tested				2526 *Klebsiella pneumoniae* isolates tested			
Antibiotic	KP Isolates tested (n)	Range of susceptibility (%)	Mean susceptibiltiy (%)	Resistant isolates n, (%)	KP Isolates tested (n)	Range of susceptibility (%,n)	Mean susceptibiltiy (%)	Resistant isolates n, (%)
Amikacin	2525	73 -100	89	271 (11)	2435	85 - 98	90.5	233 (9.5)
Piptazobactam	2497	34 - 83	63.8	963 (36.2)	2456	35 -79	55.2	1148 (44.8)
Cefotaxime	2506	16 - 41	32	1763 (68)	2329	10 - 39	27.2	1714 (72.8)
Cefepime	2303	16 - 42	31	1624 (69)	2292	13 - 36	27.2	1697 (72.5)
Ertapenem	1990	93 - 100	98	44 (2)	2014	91 - 100	97.3	84 (2.7)
Imipenem	2307	97 - 100	99	13 (1)	2288	94 - 100	98.4	57 (1.6)
Meropenem	2557	97 - 199	99	16 (1)	2461	93 - 100	98.5	63 (1.5)

KP - *Klebsiella pneumoniae*
BSI – bloodstream infection

During a 2-year review, 2002-2003, at the neonatal unit at Chris Hani Baragwaneth Hospital in Johannesburg, a central referral centre in Gauteng, reviewers found 100 neonates with ESBL-KP BSI and or ESBL-KP meningitis. From the available data on blood cultures taken at 48 hours after commencing the empiric combination therapy of piptazobactam and amikacin, about 47% (9/19) of isolates were resistant to this combination, necessitating a change to meropenem. Low birth weight (LBW) infants with *KP* BSI and meningitis had a high (30%) mortality rate, particularly if the illness involved respiratory failure and inadequate empiric therapy. The authors concluded by recommending carbapenem antibiotics for the treatment of all invasive ESBL-KP infections (Velaphi et al., 2012).

In 2009, a 1-year review of BSIs at the neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital in Gauteng revealed 246 BSIs in 181 infants. Researchers found KP, the second most common isolate after *Staphylococcus epidermidis*, to cause high mortality (29%) in infants. Of the KP isolates, 65% were ESBL-producers. Whilst all the *E Coli* isolates were sensitive to 3rd generation cephalosporins and aminoglycosides, 94% and 83% of KP isolates were susceptible to amikacin and ciprofloxacin respectively. Mortality was significantly higher in neonates with gram-negative infections 32/111 (28.8%) compared to those with gram-positive infections 14/135 (10.4%) ($P < 0.001$) (Ballot et al., 2012).

1.6 *Klebsiella pneumoniae* infections in the study setting: Red Cross War Memorial Children's Hospital

Since 2011, *KP* has become the dominant nosocomial bacteraemic agent at RCWMCH (Lochan et al., 2013). RCWMCH is a multidisciplinary tertiary hospital dealing with not only children with complex medical conditions but also admitting children with diseases of poverty into in the level 2/3 functional area of the hospital. Poverty

diseases include malnutrition, diarrhoeal disease, respiratory infections, and tuberculosis.

Researchers conducting a study reporting on the bloodstream pathogens at the institution over 5 years, from 2008 to 2012, found that Gram-negative organisms made up 57.9% (1679/2969) of isolates. KP was the most common *Enterobacteriaceae* and its prevalence showed significant reduction over the 5 years from 45.8% in 2008 to 31.7% in 2012 (Lochan et al., 2013).

Researchers recently described *KP* as the most common cause of post kidney transplantation UTI at RCWMCH, which may reflect the institutional range of microbiota as well as the powerful immunosuppression used. Sixty two children with a mean age of 10 years were followed up after kidney transplantation for a mean period of 36.9 months. Of these children, 40% developed UTI with KP as the most common causative organism (Esezobor et al., 2012).

Hospital mortality statistics reflected the importance of studying hospital-acquired infections. A 2008 study on ventilator-associated pneumonia (VAP) at RCWMCH involving 230 paediatric intensive care unit (PICU) children aged 3.9 (IQR 2.2-9.1) months where 25% were HIV-infected, showed that after *Acinetobacter baumanii, KP* was the second most common VAP-defining isolate from bronchoalveolar lavage specimens. KP was responsible for 28.8% (15/52) of cases. Of all the study children, 40 (17.4%) died during PICU admission, and a further 15.2% (n=35) died later (Morrow and Argent, 2009).

1.7 Risk factors for KP BSI and ESBL-producing KP BSI in children

Researchers have not clearly defined factors predisposing hospitalised children to bloodstream KP infections for African children. However, risk factors that were

identified in other geographical settings may also influence the risk of acquiring KP BSI in African children. A recent American paediatric study identified 3rd-generation cephalosporin use in the previous 30 days as a significant risk factor for ESBL-producing KP infection (Zaoutis et al., 2005).

A 5-year review in Korea involving 157 blood isolates from children showed prior hospitalisation, PICU admission in the previous month, and prior exposure to 3rd generation antibiotics as risk factors for acquisition of ESBL-producing *Enterobacteriaceae.* The study population had similar clinical features. However, mortality was higher in children with ESBL-producing strains of *E Coli* and KP compared with those who had non-ESBL-producing isolates, 12 of 45 (26.7%) versus 5 of 87 (5.7%) (P = 0.001) (Kim et al., 2002).

A 2-year prospective study on febrile neutropaenic cancer patients in Kuala Lumpur, Malaysia showed KP as the causative agent in BSI in 29 children. This accounted for 44% of all *Enterobacteriaceae* BSI in a paediatric oncology unit where leukaemia and lymphoma were the two most common underlying cancers in 18 and 4 children respectively. The mean age was 76.9 months. Hospitalisation for longer than 2 weeks and exposure to 3rd generation cephalosporins within 2 weeks prior to presentation were significantly associated with presumed ESBL-KP infection in a multivariate analysis: adjusted odds ratio (aOR) 14.9(95% CI 2.3-99.6) and aOR 11.1(95%CI 1.3-95.2) respectively. Additionally, researchers associated delays in effective antibiotic treatment with increased mortality, OR 9.9 (95%CI 1.5-52.3) (Ariffin et al., 1999).

In neonates, researchers described risk factors such as low birth weight, length of stay (LOS), duration of TPN, prolonged ventilation(>7days), and exposure to any antibiotics in the univariate analysis. Duration of TPN was the only independent

multivariate risk factor for acquisition of Gram-negative BSI including *Klebsiella spp*. BSI (Samanta et al., 2011).

A clinical study in adults with KP BSI indicated that the presence of a CVP catheter and mechanical ventilation are significant adjusted risk factors for ESBL KP (Tuon et al., 2011). In another study, univariate analysis identified dialysis, solid-organ transplantation, chronic liver disease, and cancer as risk factors for acquiring KP BSIs in a population-based study and were associated with a 20% mortality rate (Meatherall et al., 2009). In contrast, researchers in a further adult study found older age group (>60years), length of hospitalisation, and prior antibiotic exposure to be significant factors associated with ESBL-KP acquisition (Tumabarello et al., 2006).

It is clear that there is limited information on paediatric *KP* BSI as well as on the risk factors for acquisition of ESBL-strain infection. Three paediatric studies have been found that specifically addressed KP BSI: two retrospective descriptive case series with limited risk factor analyses and one prospective descriptive study on febrile neutropaenic children with cancer. Together, these studies involved 186 children with KP BSI (see Table 1.5).

Table 1.5: Risk factors for ESBL-strain acquisition in paediatric studies involving Gram-negative (including *Klebsiella pneumoniae* (KP)) bloodstream infections and crude mortality rates

Study reference	Country	Study period	Age range	Study design	N (cases)	Identified risk factor for ESBL-strain acquisition	Mortality (crude)
Zaoutis et al., 2005	USA	1999-2003	0-11y	Case-control ESBL-EC and ESBL-KP BSI	35:105	Exposure to 3rd generation cephalosporins -female gender - previous steroid use	36%
Kim et al., 2002	Korea	1993-1998	0-17y	Retrospective descriptive ESBL-EC and ESBL-KP BSI	142	-Prior hospitalisation -Use of 3rd generation cephalosporins -PICU admission	12.9%
Samanta et al., 2011	U.K.	1999-2005	neonates	Retrospective case-control. GN BSI and meningitis	48:96	Duration of TPN	27%
Tsai et al., 2014	Taiwan	2004-2011	neonates	Prospective descriptive on GN BSI	333	Prior exposure to 3rd generation cephalosporins -underlying renal disease	16%
Bonadio, 1989	USA	1979-1988	2-19y	Retrospective descriptive on KP BSI	57	N/A	20%
Ariffin et al., 1999	Malaysia	1996-1997	7m-12y	Prospective descriptive KP BSI in febrile neutropaenia	29	Hospitalisation and prior exposure to 3rd generation cephalosporins	34.5%
Velaphi et al., 2009	RSA	2002-2003	neonates	Retrospective ESBL KP BSI and meningitis	100	N/A	30%
Levy et al., 1996	Israel	1988-1994	0-18 y	Prospective descriptive on GN BSI	304	Previous AB therapy, CVP, previous corticosteroid therapy, previous cytotoxic therapy, neutropaenia	11%

ESBL-EC: ESBL-producing *Escherichia Coli*
ESBL-KP BSI: *ESBL-Klebsiella pneumoniae* bloodstream infection
GN BSI: Gram-negative bloodstream infection including Klebsiella
CVP: central venous catheter
AB: antibiotic
N/A: not analysed

1.8 Laboratory identification

Effective management of a child with a KP BSI requires rapid recognition with timeous and correctly taken blood cultures followed by the laboratory's rapid and accurate detection and identification of the organism. In Cape Town, South Africa, this is accomplished through the use of an automated system known as Vitek®2, to rapidly identify bloodstream infection coupled with disc diffusion susceptibility testing following international standards and guidelines. (Bamford C, personal communication) Detailed descriptions of the microbiological methods are given in section 2.4: Laboratory procedures.

1.9 Treatment

Healthcare providers should institute rational empiric antibiotic regimens early, before the laboratory results have been finalised, since delays or incorrect antibiotic choices are associated with increased mortality (Schwaber and Carmeli, 2007). During the study period, commonly chosen antibiotic combinations for a suspected nosocomial infection at RCWMCH were either a 3rd generation cephalosporin such as ceftriaxone or cefotaxime in combination with amikacin, an aminoglycoside, or piptazobactam in combination with amikacin. The present study should indicate whether these combinations remain appropriate for bloodstream KP infections at this institution. During the study period, healthcare providers commonly used ampicillin and gentamicin as empiric antibiotic therapy for all children with suspected community-acquired BSI, including children with severe or acute malnutrition. The high mortality reported in patients with bloodstream KP infections lends urgency to strengthening antibiotic stewardship and a surveillance system that would provide current and appropriate treatment guidelines.

1.10 Rationale for this research project

The above literature review indicates that BSI caused by KP may constitute a serious health problem for affected children with a high mortality risk. There are, to date, no comprehensive studies specifically describing the burden of hospital acquired KP BSIs in South African children.

At RCWMCH, researchers found *Enterobacteriaceae* to account for 64% (1 074/1 679) of all Gram-negative pathogenic isolates and 36.2% (1 074/2 969) of all pathogens in an audit of blood cultures taken over a 5 year period, 2008-2012. Of the *Enterobacteriaceae, KP* was the dominant species causing BSI (41.8%). On a positive note, the annual proportion of infections caused by KP isolates have shown a steady decline from 45.8% in 2008 to 31.7% in 2012 ($\chi 2$ for trend p=0.004) (Lochan et al., 2013).

Healthcare providers neither provided screening nor practised universal contact precautions at this institution. Poor infection control practice, especially suboptimal application of contact precautions including hand washing or by seasonal overcrowding such as is common in the RCWMCH short stay ward (SSW), may be largely responsible for the dissemination of resistance plasmids. Sick patients may be referred from the SSW for admission to the wards in the main hospital or those at other district or secondary hospitals in Cape Town. Overcrowding increases the susceptibility of the healthcare setting to heavy contamination. In the SSW, patient cots are often placed too close together with less than an arm's length between them; several children may share the resuscitation couches in the emergency room at any one time. These practices occur in order to accommodate high numbers of children, but in so doing commonly lead to a breakdown in infection control practices. Overcrowding and increased patient to healthcare staff ratios throughout the hospital

often accompanied overcrowding in the SSW. High numbers of patients can even pressure surgical wards into accepting non-surgical patients in order to accommodate high numbers of sick children during seasonal caseload surge conditions.

Managing patients with nosocomial infections is expensive and challenging with respect to treatment options and patient survival. The cost of an uncomplicated stay in a general ward bed at this children's hospital during the study period was R2000 a day; stay in the PICU costs at least 4 times more (RCWMCH Hospital electronic patient Data system, CliniComTM Application Manager, 2012). Healthcare providers may expect children with healthcare associated sepsis to require considerably more resources than the admitting diagnosis predicted and suffer considerable morbidity.

From what has been written on the epidemiology of paediatric KP BSI, the knowledge outcomes are severely limited. This paves the way for an in-depth analysis on the subject. Researchers have not evaluated the clinical profile of children with KP BSIs at this institution. The focus of this project is to explore the problem of laboratory confirmed KP bloodstream infections (LCBSI) in hospitalised children by linking patient demographics, clinical data, antibiotic resistance patterns, and clinical outcome in order to describe the emerging problem of multi-drug resistant *KP* BSI at RCWMCH.

Additionally, a specific study objective was to uncover potential predisposing factors unique to hospitalised South African children in order to improve awareness and highlight the gravity of the outcome of hospitalised children that acquire nosocomial KP infections. By describing the antibiotic resistance profiles, the hope is to provide some clarity to rational antibiotic choices. Healthcare providers could also use the information thus generated to direct feasible preventive strategies once they know

potential risk factors. This information may also be useful in developing effective treatment strategies at this hospital and could inform other state hospital practices. In a parallel study, researchers recently showed the effect of methicillin resistant *staphylococcus aureus* (MRSA) at RCWMCH to account for 53% of 32 deaths attributed to *Staphylococcus aureus* BSI in a 5-year epidemiological review of staphylococcus BSIs. The authors of this study documented 365 cases of *Staphylococcus aureus* BSI over a 5-year period, 2008-2012. Multidrug resistant MRSA caused 26% (95/365) of the cases and was the only significant risk factor for mortality. Malnutrition, residents of intermediate care health facilities, and age of less than 12 months posed the greatest risk factors for acquisition of MRSA BSI (Naidoo et al., 2013). The clinical significance of the results of this study was substantial. Healthcare providers used the information to improve patient outcomes by improving empiric therapy and avoiding delays in therapy initiation for children with staphylococcal BSIs. Finding the appropriate antibiotic cover for healthcare- associated KP BSI would further enhance patient care at the institution.

1.11 Aim and objectives

1.11.1 Main aim

The main aim was to describe the emerging problem of multi-drug resistant *KP* BSIs at RCWMCH.

1.11.2 Specific objectives

(1) The first specific objective was to describe the presentation of *KP* BSIs at RCWMCH over a 6-year period.

(2) The second objective was to describe the spectrum of risk factors associated with ESBL- *KP* BSI.

(3) The third objective was to compare health-care related and community acquired *KP* BSI.

(4) The fourth objective was to evaluate the antibiotic susceptibility patterns of *Klebsiella pneumoniae* isolates and the antibiotic selection and response to KP-blood stream infections

(5) The fifth objective was to evaluate cause of death and factors associated with inpatient mortality.

(6) The final objective was to assess the outcome of *KP* BSI in HIV-infected and uninfected children.

CHAPTER TWO: METHODS

2.1 Study design and setting

This study was a hospital-based, retrospective case folder review conducted at Red Cross War Memorial Children's Hospital (RCWMCH) from 1 January 2006 to 31 December 2011.

RCWMCH is a public sector hospital for children aged 0-13 years; it is also an academic teaching hospital. Doctors assessed an average of 3500 new children per month (range 3000 to 6000 per month) in the medical emergency unit and hospitalized up to 23 000 children per year. It is one of the two local tertiary referral units servicing 1.5 million children under 14 years of age in the Western Cape. (STATS SA, 2011) It also serves as a national referral centre for children with selective sub-specialist problems. The hospital has a 20-bed multidisciplinary PICU, an 18-bed burn unit, a 17-bed Oncology unit, 60 mixed surgical beds, 58 general paediatric beds, 29-bed ward for cardiology and tracheostomy patients, 29 sub-specialty medical beds, 10-bed trauma unit, and 42 short stay medical beds. Admission numbers peak during seasonal diarrhoeal months, January to April, and overcrowding in the SSW becomes the norm with staff accommodating up to 90 children in this ward. Many children requiring admission into the main hospital beds frequently start their hospital stay in the SSWs. The main entry points into the hospital are via the medical emergency or trauma units.

2.2 Study population

Most of the children admitted to this hospital came from homes in low socioeconomic peri-urban settlements in the Western Cape Province. Some admissions were inter-hospital transfers from neonatal units, secondary, and district level hospitals within

the province. The Medical Microbiology laboratory of the National Health Laboratory Service (NHLS) identified and forwarded an electronic list of all children hospitalised at RCWMCH with *Klebsiella pneumoniae* (KP) bloodstream infection (BSI) from 1st January 2006 to 31st December 2011.

2.3 Data collection

The first part of the review focused on hospital case notes of all the children who experienced KP BSI. Data included paper-based hospital case notes including patient demographics, age determined from the date of birth and current hospital admission, and HIV and nutrition status. These also included medical information including admission diagnosis, underlying medical conditions, previous medical history, clinical information regarding the BSI, antibiotic administration, potential risk factors for ESBL-producing BSI and mortality, length of hospital stay, and outcomes such as whether individual patients died during the current admission or discharged alive. Part of the extracted information included routine laboratory information from the NHLS database: full blood count (FBC) and differential, C-reactive protein (CRP), procalcitonin (PCT), urea and electrolytes, serum creatinine, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen level, blood gas, alanine and aspartate aminotransferase, and serum bilirubin. A standardised data sheet was used to compile the information (see Appendix A).

A clinician, Heloise Buys (HB), and a nursing sister affiliated to the paediatric infectious diseases unit (PIDU), Spasina King, captured all data on standardised data sheets. HB subsequently entered into an Excel electronic database. The data sheets and electronic database are contained within a locked office belonging to HB.

2.4 Laboratory procedures

All the microbiological tests in this study were performed in an accredited NHLS laboratory. The laboratory utilised the automated BacT/ALERT® blood culture system. For blood culture bottles flagging positive with mono-morphic Gram-negative bacilli seen on Gram stain, a locally validated method of direct inoculation into the automated Vitek®2 system (bioMérieux, Inc, France) was used (Bamford et al., 2010). Based on the method described by Bruins, this involved separation of bacteria by centrifugation at 1525g for 10 minutes of a 4ml aspirate from the positive blood culture bottle in a serum separator tube. Following removal of the resulting supernatant, the film of bacteria sitting on top of the gel layer was re-suspended in 3ml of 0.45% saline to a density of 0.6 – 0.8 McFarland standard. Vitek ID-GNB and AST-N064 cards were used for identification and susceptibility testing respectively (Bruins et al., 2004).

The current Clinical and Laboratory Institute (CLSI) criteria helped to interpret antimicrobial susceptibility results (CLSI, 2012). If necessary, this also included alternative identification and susceptibility methods, including biochemical reactions, disc diffusion susceptibility testing, Etest minimum inhibitory concentration (MIC) testing, and further Vitek testing performed from bacterial colonies subcultured onto agar plates.

ESBL production was inferred based on MICs/growth rates of various cephalosporins with/without inhibitors either via the Vitek 2, or by double disc synergy testing. If ESBL production was detected, all cephalosporins, excluding cephamycins, were reported as resistant, irrespective of MIC or zone size. This study reported all known cephalosporins, excluding cephamycins, as resistant if detecting ESBL production,

irrespective of MIC or zone size. Specific reporting or documentation in laboratory information system of ESBL production has been quite inconsistent nationally. Therefore, for surveillance purposes, the NHLS laboratory relied on the pattern of antibiotic resistance and assumed that resistance to cefepime was equivalent to presence of ESBL for *Enterobacteriaceae*. The Vitek expert system infers presence of ESBL based on MICs or growth rates of various cephalosporins with or without inhibitors.

2.5 Case Definitions

- *Healthcare-associated infection(HAI):*

A community-onset *Klebsiella pneumoniae* blood stream infection confirmed on a blood culture specimen that was obtained within 48 hours of hospital admission in a patient with 1 or more healthcare-associated risk factors (HRFs).

  - *Healthcare-associated risk factors (HRFs)*
  - Presence of an invasive medical device e.g., vascular catheter
  - Surgery, hospitalisation, dialysis or residence in an intermediate healthcare facility in the preceding 12months of admission
  - History of KP infection or colonization (adapted for this study)

- *Nosocomial infection*

A blood stream infection was deemed nosocomial if *Klebsiella pneumoniae* was confirmed on a blood culture specimen that was obtained more than 48 hours after admission to hospital, or within 48 hours of discharge from hospital, with or without HRFs (Klevens et al., 2006).

- *Community-acquired infection (CAI)*

A *Klebsiella pneumoniae* blood stream infection confirmed on a blood culture specimen that was obtained within 48 hours of admission to hospital, with no associated HRFs.

- *Healthcare-related infection*

All children who met the definition of either healthcare-associated or nosocomial BSI

- *Presumed ESBL-producing KP (ESBL KP)*

Based on the pattern of antibiotic resistance at the NHLS laboratory, for *Enterobacteriaceae* including *Klebsiella pneumoniae* isolates, resistance to cefepime was inferred equivalent to presence of ESBL. In this document presumed-ESBL-producing KP will be referred to as ESBL-KP.

- *Acute diarrhoea*

The production of 3 or more loose stools per day for less than 14 consecutive days (WHO, 2005).

- *Chronic diarrhoea*

The production of 3 or more loose stools per day for a minimum of 14 consecutive days (WHO, 2005).

- *HIV infection*

A positive HIV DNA PCR result in any child < 18 months old, or 2 positive serological test results (HIV ELISA or HIV Rapitest) or a positive HIV DNA PCR result in a child > 18 months old are considered HIV-infected (Centers for Disease Control and Prevention, 2008).

- *Unknown HIV status*

Any infant of child where there was no record of HIV testing at the NHLS laboratory database and whose mother's HIV status was unknown.

- *HIV-exposed status*

Any infant of child where there was no record of HIV testing at the NHLS laboratory database and whose mother's HIV status was known to be positive.

- *Definition of severe immunosuppression by CD4 lymphocyte percentage and count*

Any HIV-infected infant or child with a percentage CD4 or absolute CD4 count below the ranges set out in the using the 2006 WHO definitions (WHO, 2006) as summarised in Table 2.1.

Table 2.1: Threshold CD4 cell count and percentage levels for severe HIV immunodeficiency in infants and children

Percentage CD4 (%)	Age months (m)	Absolute CD4 count cells/mm^3
<25	< 11 m	<1500
<20	12 - 35 m	<750
<15	36 - 59 m	<350
<15	>60 m	<200

- *Nutritional status*

  Moderate and severe underweight were classified as weight-for-age z score (WAZ) between -2 and -3 standard deviations (SD) below the median using World Health Organisation (WHO) growth reference standards, and a WAZ < -3 SD respectively. Weight-for-age z-scores were calculated from the 2000 Centers for Disease Control and Prevention (CDC) Growth Reference Standards in the United States using the STATA command: egen zwaus= zanthro(wa, us), from "Standardizing anthropometric measures in children and adolescents with new functions for egen" (Vidmar et al., 2004).

- *Polymicrobial BSI*

  A BSI in which *Klebsiella pneumoniae* and one or more bacterial pathogens other than *Klebsiella pneumoniae* were isolated from a single blood culture specimen.

- *Laboratory investigations*

Reference is made in this document to laboratory investigation results; with the exception of the HIV testing and the CD4 lymphocyte counts, these results were captured if the tests were taken on the same day as or within 48 hours of the *KP* blood culture. In the case of the CD4 lymphocyte counts the most recent result was captured; in South African state institutes CD4 lymphocyte monitoring is done at baseline and then at 6-12 monthly intervals (Meyers et al., 2005). In this study the latest recorded CD4 count did not necessarily correspond with the timing of the BSI but in all cases where documented fell within the preceding 3 month period.

- *Haematological indices*
    - Anaemia was defined as a blood haemoglobin level less than 11g/dl (WHO, 2011).
    - Leukopaenia was defined as a white blood cell count below the reference range for age.
    - Thrombocytopaenia was defined as a platelet count < 150 × 10^9/L**.**
    - Bicytopaenia was defined when there was reduction in two of the three main blood cell lines.
    - Pancytopaenia was defined by the reduction of the three formed elements of blood below the normal reference ranges for age i.e., anaemia :haemoglobin ,11g/dl; thrombocytopaenia: platelet count <150 x 10^9/L and leukopaenia: white blood cell count < age-related reference range

    - Coagulopathy was defined as an elevated out of age range prothrombin time (PT) of ≥ 2seconds; or an activated partial thromboplastin time (APTT) of ≥60s or a depressed fibrinogen level of <2 μmol/L (Goldstein et al., 2005 and Angus et al., 2013)

- *Infective biomarkers*
    - Procalcitonin (PCT) was considered elevated if the value was >0.5 μg/L
    - C-Reactive protein (CRP) was considered elevated if the value was >10 mg/dL

Other organ dysfunctions

- *Liver dysfunction*

  Where measured, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin were used as biomarkers of liver dysfunction. Specifically dysfunction was assumed when one or more of the liver enzymes markers were elevated to twice or more of their normal values or the bilirubin was >70µmol/L (excluding new-born infants) (Goldstein et al., 2005 and Angus et al., 2013).

- *Renal dysfunction*

  Where measured, the serum creatinine expressed in µmol/L was used as a biomarker of renal dysfunction. In the current study renal dysfunction was defined as a serum creatinine above the upper limit (>97.5th percentile) for paediatric age group. Renal function assessment is now commonly performed by assessing the estimated glomerular filtration rate (eGFR) which is further dependent on gender, height and creatinine clearance. From this a determination of whether kidney injury (previously called renal impairment/ dysfunction) is present can be made. In our study patient heights were not routinely documented in the hospital case notes during the admission periods and hence eGFR could not be determined (expressed in ml/min/1.73m^2), additionally, as nearly two thirds of children were malnourished in terms of weight-for-age z-scores, a default assumption of normal height could not be used to estimate the GFR. There is evidence that serum creatinine measurements still provide useful assessment of renal function and may be more appropriate in young children (Pottel et al., 2008, and Boer et al., 2010). At RCWMCH's clinical chemistry

laboratory serum creatinine is determined using an enzymatic assay method in line with international recommendations and the results referenced against age-matched reference ranges Lewington and Kanagasundaram, 2011).

- *Septic shock*

  Carcillo described septic shock as a state of tachycardia with feeble peripheral pulses, capillary refill time >2seconds, reduced level of consciousness, mottled or cool hands and feet, and reduced urine output (Carcillo, 2003).

  On the other hand according to the Surviving Sepsis Campaign the presence of sepsis together with haemodynamic inadequacy: the need for inotropic agents to maintain a normal blood pressure (BP) for age; or the presence of hypotension (systolic BP<2 SD below normal for age); or 2 or more of: metabolic acidosis with a base deficit of > 5mEq/L; blood lactate >4; capillary refill time >5 s; oliguria, core-toe temperature gap of more than $3^{o}C$ constitute septic shock (Goldstein et al., 2005 and Dellinger et al., 2012) However, in this retrospective study, the diagnostic criteria, clinical and treatment decisions made by the attending clinicians under the guidance of specialists stood, and their documented assessment of sepsis and shock were used to define a binary parameter of whether there was septic shock present or not.

- *Respiratory failure*

  Respiratory failure was simply defined as the need for mechanical ventilatory support.

- *Excoriated skin*

  Excoriative skin lesions , including burn wounds, that were significant enough to merit documentation in the medical or nursing notes were captured as a binary parameter: *excoriated skin* or not. Skin failure is a recognised dermatological entity describing a loss of skin integrity interfering with the barrier protection mechanism against mechanical damage and microbial penetration as well as other skin functions such as temperature regulation, fluid, electrolyte and protein retention. (Irvine, 1991)These functions are not directly amenable to laboratory testing but assessed visually and described by area, extent, site and depth of skin necrosis where possible.

2.6 Data analysis

Using the patient identifiers obtained from the microbiology database, clinical and laboratory data were extracted retrospectively from the hospital folders, and together with antibiotic sensitivity results obtained from the GSH microbiology database, entered on a standardised data collection sheet (Appendix A). The data was entered anonymously in an Excel spread sheet by a single investigator (HB) and analysed using STATA Statistical software, release 11, (College Station, Texas, USA).

Firstly, conventional descriptive methods, mean ± SD or median [interquartile range], were used to describe the dataset. The Shapiro-Wilks method helped with evaluating the distribution of continuous variables for normality. The non-parametric analytic methods, Kruskal-Wallis equality of populations' rank test or Wilcoxon rank-sum test for independent samples, then helped to compare continuous variables. These variables included the median age of children who died versus that of those who survived and the Chi-squared or Fisher exact test used to compare categorical variables. The Wilcoxon rank-sum test helped to compare medians within two groups where data were continuous.

Risk factor analysis

Univariate analysis helped to identify factors that were possibly associated with the risk of *ESBL-KP infections*. The subsequent multivariate analysis included variables with a p-value <0.1 using generalised linear modelling using Poisson regression with robust error variance to explore and quantify their relationships while correcting for confounders. These variables included gender, age, nutrition status, HIV infection, exposure to antibiotics, chronic underlying medical conditions, and ward management exposures such as intravenous (IV) fluid infusions, central vascular catheters, ventilation, and surgery. Given the high prevalence of ESBL-associated infections (83%), Risk Ratios with Confidence Intervals (CI) of 95% helped to quantify the measure of effect between groups after controlling for confounders.

Univariate analysis helped to identify factors that were possibly associated with risk for *mortality,* and the subsequent multivariate analysis included any variables with p-values <0.1 using generalised linear modelling. These variables included gender, age, nutrition status, HIV infection, exposure to antibiotics, chronic underlying medical conditions, and ward management exposures such as IV infusions, ventilation, and surgery. Given the high prevalence of mortality (30%), Risk Ratios with Confidence Intervals (CI) of 95% helped to quantify the measure of effect between groups after controlling for confounding factors. Additional sub-analyses proved useful when exploring differences in the group of children that died early, within 3 days and within 14 days of the bacteraemic event, and later in the admission, >14 days after the BSI, as well as the group of children who presented with diarrhoeal disease.

Both the univariate and multivariate analyses included using a significance level of p< 0.05; 95% confidence intervals were quoted where relevant. The basis for infection rates was infections per 1000 hospital admissions.

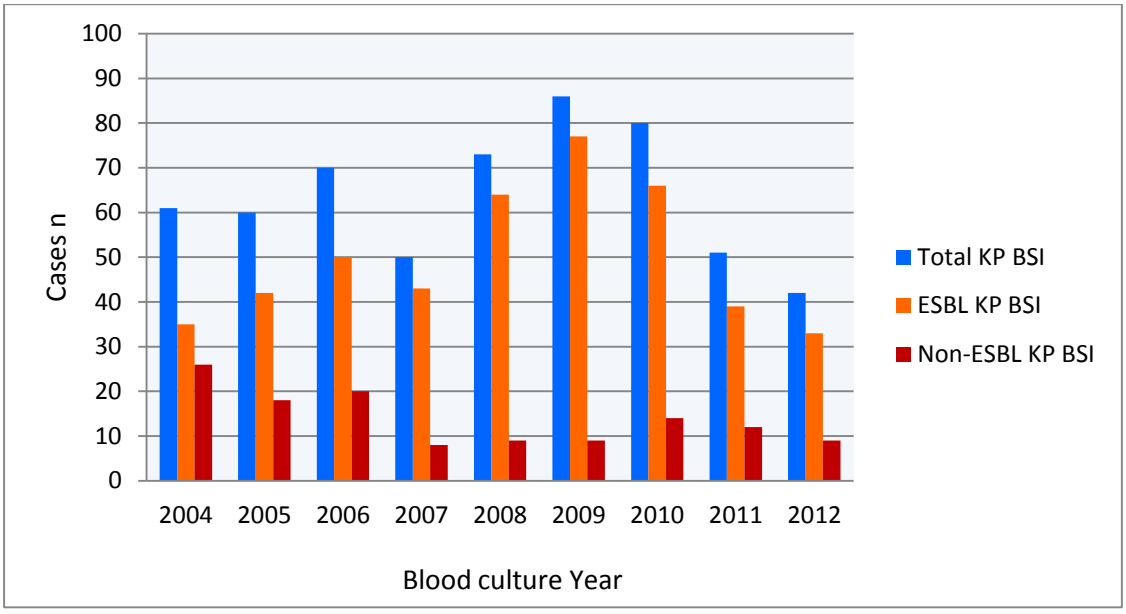
2.7 Ethical issues

The study was approved by the Health Sciences Faculty Research Ethics Committee of the University of Cape Town (HREC REF: 367 /2009) and the Research Committee of RCWMCH. The study was done in accordance with the Declaration of Helsinki, (2008). Since the researchers collected data retrospectively, individual consent was not obtained from parents/legal guardians. The data sheets included the folder numbers of patients to enable the researchers to check information from the folders after data collection was completed. Each folder number was linked to a study number. Study numbers (but not names) were entered on an electronic database for anonymous analysis and reporting. The paper-based data sheets were kept in a locked office (HB) and the electronic database was encrypted using a password and available only to HB.

CHAPTER THREE: RESULTS

The total number of KP BSI, the number of presumed ESBL KP BSI and the number of non-ESBL BSI per annum for the period 2004 until 2012 at Red Cross War Memorial Children's Hospital (RCWMCH) is shown in Figure 3.1. The present study, which analyses KP BSI during the period 2006-2011, is nested within this graphical analysis.

Figure 3.1: *Klebsiella Pneumoniae* bloodstream infections at RCWMCH January 2004- December 2012

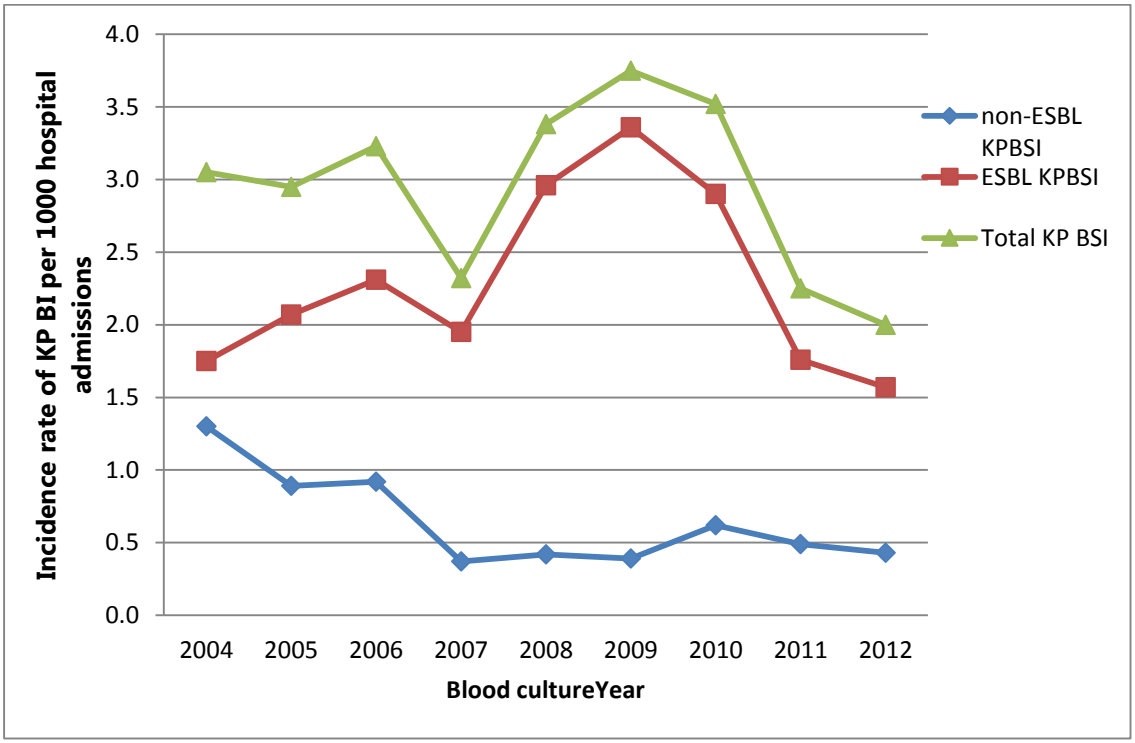


RCWMCH: Red Cross War Memorial Children's Hospital
KP BSI: *Klebsiella pneumoniae* bloodstream infection
ESBL KP: extended-spectrum β-lactamase producing *Klebsiella pneumoniae*

3.1 Incidence rates of ESBL KP and non-ESBL KP BSI

The annual incidence of KPBI in 2006 was 3.23 cases per 1000 hospital admissions with a mean frequency of 2.54 ESBL and 0.54 non-ESBL cases per 1000 hospital admissions per year. The incidence of ESBL-KP infections increased from 2007 to peak in 2009 and then declined to 1.76 cases per 1000 in 2011. The incidence of non-ESBL KPBI has declined from an incidence of 0.92 cases per 1000 to 0.43 over the study period (see Figure 3.2).

Figure 3.2: Incidence of ESBL- and non ESBL-producing *Klebsiella pneumoniae* bloodstream infections at RCWMCH 2004-2012



ESBL-KP BSI: extended-spectrum β-lactamase producing *Klebsiella pneumoniae* bloodstream infection

3.2 Characteristics of the study population

Over the 6 year study period, January 2006 to December 2011, 410 hospitalised children had laboratory confirmed KP BSI; 339 (83%) of these were ESBL-related infections. The median age of the 410 children was 5.0 months, interquartile range (IQR) 2 to 16 months with 68.9% (282) being less than 12 months of age. There were 212 males and 198 females. Of all children, 33 (8%) resided in intermediate healthcare facilities.

Demographic information was complete for all 410 children and clinical data was complete for 407 children (see Table 3.1). Table 3.1 displays the results comparing and exploring possible differences between BSIs that were ESBL versus non-ESBL related.

Table 3.1: Baseline characteristics of the children with *Klebsiella pneumoniae* bloodstream infection

Variable	All	ESBL-KP	Non-ESBL-KP
Number of children (%)	n=410	n=339 (83%)	n=71 (17%)
Male: female	212: 198	170: 169	42: 29
Median[IQR] age in months	5 (2-16)	5 (2-5)	10 (2-29)
Median weight-for-age z-score (IQR)	-2 (-4 to -1)	-2 (-4 to -1)	-1 (-3 to -1)
Moderate under-weight-for-age n/N (%)	78/410 (19.0)	66/339 (19.5)	12 /71 (16.9)
Severe underweight-for-age n/N (%)	163/410 (39.8)	143/339 (42.2)	20 /71 (28.2)
HIV-infection* (%)	(n=288) 82 (28.5%)	(n=249) 79 (31.7%)	(n=39) 3 (7.7%)

KP: *Klebsiella pneumoniae*
IQR: interquartile range
n/N: stratum specific proportions
*HIV prevalence known in 288 children

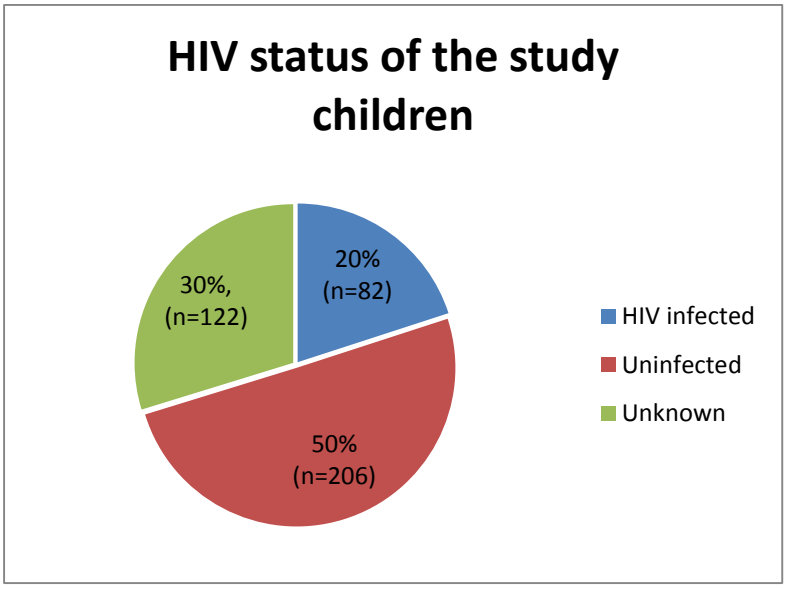
3.2.1 Nutritional status

Regarding nutritional status, 241 (58.8%) children were moderately or severely underweight-for-age (UWFA) with 163 (39.8%) children severely UWFA. The median weight-for-age z-score (WAZ) was -2 (IQR -3.4 to -0.9). This study could not include height-for-weight and weight-for-height assessments since healthcare providers did not routinely document height in the hospital case notes during the admission periods. There were more children with ESBL-KP BSI [n=209/339 (61.7%)] who had moderate or severe underweight for age than those who had non-ESBL KP BSI, [n=32/71 (45.1%); p=0.01; RR 1.37 (95%CI 1.04-1.79)].

3.2.2 HIV status

Regarding HIV status, 206 (50.2%) children were HIV negative, 82 (20%) were HIV-infected, and the HIV-status was unknown in 122 (29.8%) children (see Figure 3.3). There were equal numbers of male and female infected-children. Compared with uninfected children, more HIV-infected children, 61 (74.4%), were moderately or severely UWFA [RR1.22 (1.03-1.44)]. HIV infection was significantly associated with ESBL infection compared with non-ESBL KPBSI on univariate analysis (Fisher's exact p =0.001, RR (RR 1.17, (1.08-1.26)). Only 3 (3.7%) HIV-infected children had non-ESBL infections. Of the present study's 82 HIV-infected children, 52/82(63.4%) presented with diarrhoeal disease, 35/82 (42.7%) presented with pneumonia, 14/82 (17.1%) presented with both.

Figure 3.3: The HIV status of the 410 study children with KPBSI

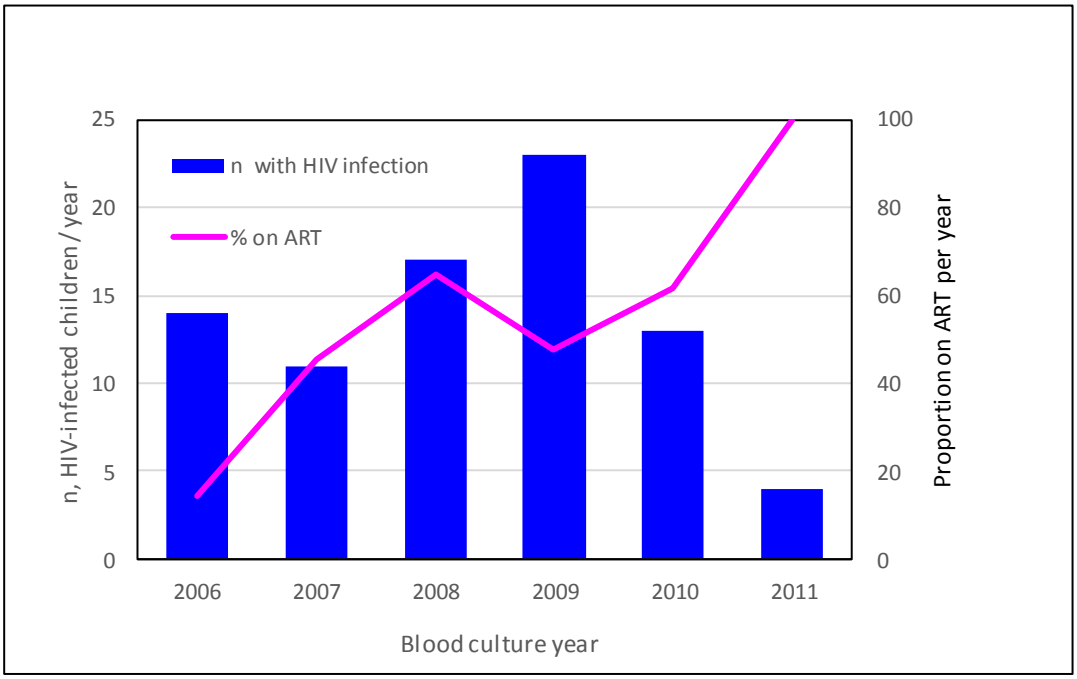


The CD4-lymphocyte count performed within 3 months of the BSI, was known in 71/82 (86.6%) of all HIV-infected children. Their median CD4-lymphocyte count was 639 (IQR 285 to 1235); their mean CD4% was 21.8% (SD ± 12.33). In this group, 42

of 71 (59.2%) children were severely immunosuppressed by CD4%-for-age, and 55/71 (77.5%) severely suppressed by absolute CD4-lymphocyte count-for-age. Of the 71 children, 55 (77.5%) were severely immunosuppressed for age by both CD4% and absolute count using the 2006 WHO definitions.

Of the 82 HIV-infected children, 41 (50%) were on antiretroviral therapy (ART) at the time of the KP BSI. The median time on ART before the KP BSI was 19 (IQR 8-66) days (see Figure 3.4). There was a progressive increase in the numbers of HIV-infected children on ART over the study period 2006-2011: in 2006 14 HIV-infected children developed KP BSI and 2/14 (14.3%) were on ART at the time of the BSI, while in 2011, 4 HIV-infected children developed KP BSI and all 4 (100%) were on ART at the time of the BSI

Figure 3.4: Number and proportion of HIV-infected children with KP BSI on ART over the study period 2006-2011

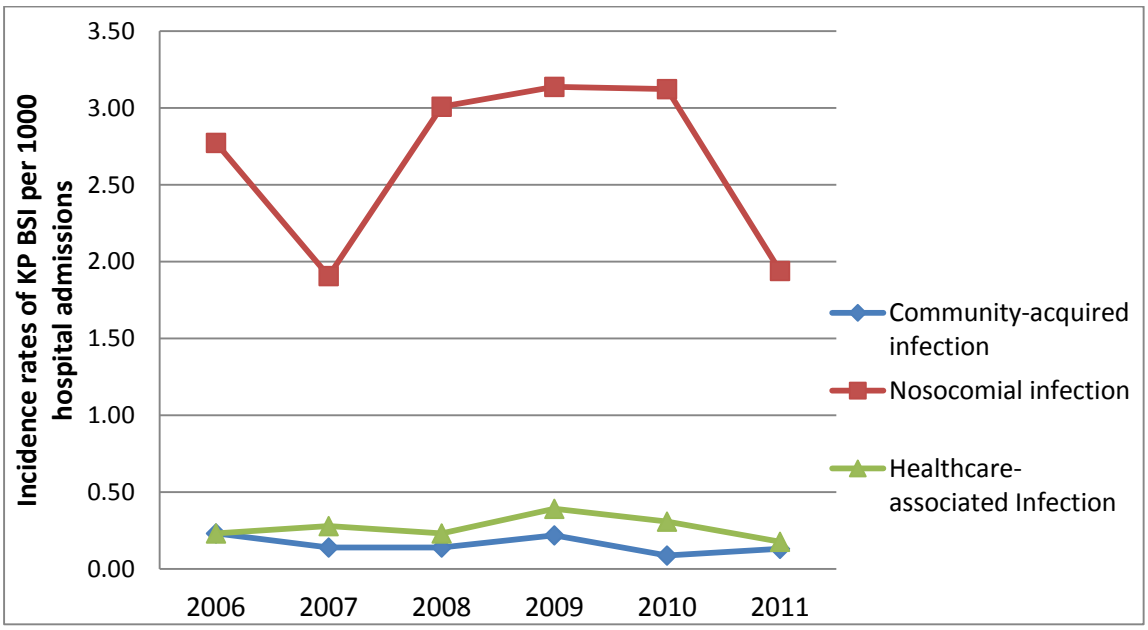


KP BSI-*Klebsiella pneumoniae* bloodstream infection ART-Antiretroviral therapy

3.3 Classification of KP BSI infections

The infection was nosocomial or healthcare-associated in 389 (95%) children and community-acquired in 21 (5%). A graph shows the trend in the incidence of community acquired KP BSI over the 6 years. The nosocomial and HAIs increased from 2007, peaking in 2009 and declining again in 2011 (see Figures 3.5). Of the children who acquired the infection within the community, 9 (2.2%) had been hospitalised within the previous 12 months.

Figure 3.5: Incidence rates of *Klebsiella Pneumoniae* bloodstream infection (KPBSI) per 1000 hospital admissions at RCWMCH January 2006-December 2011



The prevalence of non-ESBL and ESBL KP BSI by year

Table 3.2 summarises the prevelance of KP-BSI infections over the study period. Throughout the study period, nosocomial BSI caused by ESBL-producing isolates predominated. Among community-acquired infections, the number of ESBL-

producing isolates remained low. Futhermore, there has been a 47% reduction in ESBL-KP cases between 2010 and 2011, from 63 cases to 37 cases.

Table 3.2: Number and percentage of non-ESBL producing and ESBL producing *Klebsiella pneumoniae* BSI within each infection category 2006-2011

	Community-Acquired Infection		Nosocomial Infection		Healthcare-associated Infection		Total
Year	ESBL KPBSI, n (%)	Non-ESBL KPBSI, n (%)	ESBL KPBSI, n (%)	Non-ESBL KPBSI, n (%)	ESBL KPBSI, n (%)	Non-ESBL KPBSI, n (%)	Total KPBSI, n (100%)
2006	3 (4.3)	2 (2.9)	46 (65.7)	14 (20)	1 (1.4)	4 (5.7)	70 (17)
2007	0	3 (6)	38 (76)	3 (6)	4 (8)	2 (4)	50 (12)
2008	3 (4.1)	0	59 (80.8)	6 (8.2)	2 (2.7)	3 (4.1)	73 (18)
2009	1 (1.2)	4 (4.7)	68 (79.1)	4 (4.7)	8 (9.3)	1 (1.2)	86 (21)
2010	0	2 (2.5)	63 (78.8)	8 (10)	3 (3.8)	4 (5)	80 (20)
2011	0	3 (5.9)	37 (72.6)	7 (13.7)	3 (5.9)	1 (2)	51 (12)
Totals	7 (1.7)	14 (3.4)	311 (76.0)	42 (10.0)	21 (5.1)	15 (3.7)	410 (100)

ESBL KP BSI: extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* bloodstream infection

BSI: bloodstream infection

3.3.1 Comparison of healthcare-related and community-acquired KP BSI in 410 hospitalised children

When comparing children who acquired the BSI in the community with children who either were in hospital or were resident in intermediate healthcare facilities, there were 389 (94.9%) children with HRF compared to 21 (5.1%) children without (community-acquired KP BSI). Children admitted with community-acquired (CA) KP BSI were significantly younger at a median age of 1.5 (IQR 0.7-3.8) months compared with children who had healthcare-related KPBSI, their median age was 5.5 (IQR 1.9-16.2) months, p-value=0.01.

3.3.2 Comparison of healthcare-related and community-acquired KP BSI

In the adjusted analysis, children with HRF were more likely to: have ESBL-KP BSI, p-value 0.001; adjusted risk ratio (aRR) 1.19 (95% CI 1.07-1.32); be resident in the PICU at the time of the BSI, p-value 0.01; aRR 1.05 (95% CI 1.01-1.09) or have a chronic medical condition, p-value 0.005; aRR 1.08 (95% CI 1.02-1.14). Infancy, however, was associated with having a community-acquired KP BSI, p-value=0.001, aRR 0.92 (95% CI 0.88- 0.96) (see Table 3.3).

Table 3.3: Comparison of healthcare-related and community acquired KP BSI

Risk factor	HRF[#] n/N (%)	CAI[$] n/N (%)	Univariate RR RR (95% CI)	p-value	Adjusted RR aRR (95% CI)	p-value
All children	389/410(94.9)	21/410 (5.1)	-	-	-	-
ESBL KPBSI*	332/389 (85.4)	7/21 (33.3)	2.56 (1.40-4.69)	<0.001	1.19 (1.07-1.32)	0.001
Age <12 months*	264/389 (67.9)	18/21 (85.7)	0.79 (0.66-0.96)	0.09	0.92(0.88-0.96)	0.001
HIV-infection	81/276 (29.4)	1/12 (8.3)	3.53 (0.53-23.21)	0.1	1.0 (0.86-0.96)	1
Moderate or severe UWFA	232/389 (59.6)	9/21 (42.9)	1.39 (0.84-2.30)	0.13	-	-
Concomitant chronic medical condition*	258/389 (66.3)	5/21 (23.8)	2.79 (1.29-6.01)	<0.001	1.08 (1.02-1.14)	0.005
BSI without focus	192/387 (49.6)	14/21 (66.7)	0.74 (0.54-1.02)	0.13	-	-
Anaemia	279/389 (71.7)	11/21 (52.4)	1.37(0.91-2.07)	0.06	1.06 (1.0-1.12)	0.05
Patient in PICU *	82/389 (21.1)	2/21 (9.5)	5.51(0.81-37.56)	0.03	1.05 (1.01-1.09)	0.01
Pneumonia at the time of the BSI	118/386 (30.6)	4/21 (19.1)	1.60 (0.66-3.93)	0.26	-	-
Excoriated skin	125/385 (32.5)	5/21 (23.8)	1.36 (0.62-2.96)	0.41	-	-

ESBL KPBSI-Extended-spectrum β-lactamase *Klebsiella pneumoniae* bloodstream infection; [#]HRF-Healthcare risk factors; [$]CAI- Community-acquired infection; UWFA-underweight for age; PICU- Paediatric intensive care unit; IQR- interquartile range; n/N stratum specific proportions; aRR adjusted risk ratio. * Factors with p-value ≤0.5 in the adjusted risk analysis. Multivariate model adjusted for ESBL KP, HIV infection, age<12 months, concomitant underlying condition, anaemia, patient in the PICU at the time of the BSI

At the time of the KP BSI, children with HRF had a significantly lower mean haemoglobin value at 9.65 ±2.18 g/dl than 10.6 ±1.89 g/dl seen in children with community-acquired KP BSI, p-value=0.02. Whilst the median White blood cell (WBC) count and percentage band counts did not differ significantly, children with HRF had a lower median platelet count of 103 (IQR 32-288) $x10^9$/l compared with 350 (IQR 193-659) x 10^9/l seen in those children whose infection was community-acquired, p-value= 0.0001 (see Table 3.4).

Table 3.4: Comparison of haematological and pro-inflammatory indices in children with healthcare-related KP BSI and community-acquired *KP* BSI at the time of the BSI

Variable	Children with HRF KP BSI[#]		Children with CAI KP BSI[$]		p-value
n/N (%)	389/410	(94.9)	21/410	(5.1)	-
Mean haemoglobin (±SD) g/dl	9.65	(±2.18)	10.6	(±1.89)	0.02
Median WBC (IQR) $x10^9$ /l count	11.7	(6.24-19.8)	15.9	(5.07-26.3)	0.32
Median platelet (IQR) $x10^9$/l count	103	(32-288)	350	(193-659)	0.0001
Median CRP(IQR) mg/l	108	(38-194)	101.5	(50-240.5)	0.82
Median PCT (IQR) ng/ml	15.3	(1.9-41.7)	-	-	-
Percentage Median band (IQR) count	14	(5-28)	15.5	(8.5-23.5)	0.89

KP BSI: *Klebsiella pneumoniae* bloodstream infection
[#]HRF: healthcare-related KP BSI; [$]CAI: community acquired KP BSI
n/N: stratum specific proportions
SD: standard deviation
WBC: white blood cell
IQR: interquartile range
CRP: C-reactive protein
PCT: procalcitonin

3.3.3 Comparison of the complications and outcome of Healthcare and CA BSI

There was no significant difference in the mortality risk in children with HRF compared to those who had community-acquired BSI; the median time to death from the time of the positive KP blood culture (IQR) days for those with HRF was 4 (1-13) days compared to 2.5 (0.5-6) days in those with CA BSI, p=0.37 . Children with HRF were more likely to experience respiratory failure but not experience renal injury (see Table 3.5).

Table 3.5: Comparison of clinical complications and outcome in children with healthcare-related KP BSI and community-acquired *KP* BSI

Variable	Children with HRF		Children with CAI		Univariate RR		Adjusted RR	
	n/N	(%)	n/N	(%)	RR (95% CI)	p-value	RR (95% CI)	p-value
Number	389/410	(94.9)	21/410	(5.1)	-		-	
Respiratory failure*	142/388	(36.6)	2/21	(9.5)	3.84 (1.02-14.45)	0.01	1.07 (1.03-1.12)	0.001
Septic shock	132/385	(34.3)	7/21	(33.3)	1.03 (0.55-1.91)	0.93	-	
Renal injury*	94/389	(24.2)	11/21	(52.4)	0.46 (0.30-0.72)	0.004	0.91 (0.85-0.97)	0.004
Hepatic dysfunction	66/389	(17.0)	5/21	(23.8)	0.71 (0.32-1.58)	0.42	-	-
Coagulation disturbance	70/121	(57.9)	3/21	(14.3)	1.16 (0.51-2.61)	0.70	-	-
Mortality	119/389	(30.6)	4/21	(19.1)	1.61 (0.66-3.93)	0.26	-	-

KP BSI: *Klebsiella pneumoniae* bloodstream infection
HRF: healthcare risk factors
CAI: community-acquired infection
IQR: interquartile range
*p value≤0.05 in the adjusted analysis

3.4 Chronic underlying medical condition

Of the study children, 64% (262) had a chronic underlying medical condition (see Table 3.6).

Table 3.6: The chronic underlying medical condition in 410 hospitalised children with KP bloodstream infection

Chronic medical condition	Number	Percentage
Nil	148	36.1
HIV disease	82	20.0
Cardiovascular	54	13.2
Gastrointestinal	50	12.2
Malignancy	26	6.3
Renal	19	4.6
Neurological	14	3.4
Tuberculosis	8	2.0
Aplastic anaemia	3	0.7
Inborn metabolic	2	0.5
Primary immunodeficiency	2	0.5
Congenital CMV disease	1	0.2

Congenital cardiac lesions made up 91% (49 of 54) of all cardiovascular conditions. Gastrointestinal conditions included a heterogeneous spectrum with the most common being congenital intestinal atresias, biliary atresia, and gastroschisis. Chronic renal failure and hydronephrosis were the main renal conditions; 19 of the 26 children with malignancy had either leukaemia or lymphoma. The remaining seven patients with malignancy had solid tumours. Ten of the 14 children with neurological disorders had spastic cerebral palsy and two had muscle disorders. Tuberculosis was deemed an underlying medical condition if the child was on anti-tuberculosis

treatment on admission to RCWMCH. There was one child with disseminated congenital cytomegalovirus infection (see Table 3.6).

3.5 Prior hospitalisation exposure of 410 hospitalised children with *Klebsiella pneumonia* bloodstream infection

Of the children, 230 (56.8%) had been hospitalised within the preceding 12 month period. By univariate analysis, they were as likely to have an ESBL-KP BSI compared to children who had not been hospitalised; however this difference was not statistically significant (see Table 3.7).

Table 3.7: Prior (up to 12 months) hospitalisation exposure of 410 hospitalised children with *Klebsiella pneumonia* bloodstream infection (univariate analysis)

	ESBL-KP n/N (%)	Non-ESBL-KP n /N (%)	p value	Risk ratio (95% CI)
Number of children	339/410 (83)	71 /410(17)	-	-
Hospitalisation within previous 28 days	146/335 (43.6)	25/71 (35.2)	0.19	1.06 (0.97-1.16)
Hospitalisation within previous 12 months	193/334 (57.8)	37/71(52.1)	0.38	1.04 (0.95-1.14)

ESBL-KP: extended-spectrum β-lactamase producing *Klebsiella pneumoniae*
n/N: stratum specific proportions

3.6 The clinical presentation on admission to hospital of study children who developed KP bloodstream infection during hospitalisation at RCWMCH

Over the six years, diarrhoeal disease and pneumonia were the two most common admission diagnoses in 137 (33.4%) and 91 (22.2%) of the study children

respectively (see Table 3.8). The other admission diagnoses formed a large heterogeneous group of conditions with no clear pattern. One hundred and eighty two (44.4%) children had more than one admission diagnosis.

Table 3.8: The admission diagnoses of study children with KP bloodstream infection (n = 410)

Top five principal admission conditions categories:	n	(%)
Diarrhoeal disease	137	(33.4)
Pneumonia	91	(22.2)
Cardiac	46	(11.2)
Neoplasm	29	(7.0)
Burn wounds	27	(6.6)

KP: *Klebsiella pneumoniae*

The median hospitalisation time from admission to non-ESBL BSI (3days) was significantly shorter than that for the acquisition of ESBL-BSI (9days), $p<0.0001$, RR 1.45 (1.21-1.74). Table 3.9 includes a summary of these findings.

Table 3.9: Time to bloodstream infection from admission date

	All n (%)	ESBL-KP n (%)	Non-ESBL-KP n (%)	p value	Risk ratio (95% CI)
Number of children	410 (100)	339(83%)	71 (17%)	-	-
Median time(IQR) to bloodstream infection in days	7 (3-19)	9 (4-23)	3 (0-7)	<0.0001	1.45 (1.21-1.74)

3.7 The clinical spectrum of KP-bloodstream infection over a 6 year period

At the time of the KP-BSI event, 206 (50.2%) children did not have a definable clinical focus of infection. Of the children, 70% (284) experienced fever of ≥38^{0}C within the 48 hours of the positive blood culture. The most common clinical problem was pneumonia in 30% of children. The range of other clinical presentations included: wound and soft tissue infections, peritonitis, necrotising enterocolitis (NEC), and urinary tract infections (UTIs). Healthcare practitioners saw in-dwelling vascular catheter-related infections and septic peripheral vascular catheter infections in 21(5.1%) and 12 (2.9%) of the children respectively (see Table 3.10).

Table 3.10: The clinical site of infection in children with *Klebsiella pneumoniae* bloodstream infection

No. with primary bloodstream infection without a definable focus	**206**	**(50.5%)**
Common sites of infection		
Pneumonia	121	(29.7)
Vascular-catheter related	21	(5.1)
Peritonitis	20	(4.9)
UTI*	20	(4.9)
Septic wound and soft tissue	24	(5.9)
NEC#	20	(4.9)
Septic drip site(+ve$ culture of KP)	12	(2.9)
Cholangitis	4	(1)
Dysentery	3	(0.7)
Typhilitis	3	(0.7)
Bacterial endocarditis	1	(0.2)
Necrotic tumour	1	(0.2)
Bowel obstruction	1	(0.2)

UTI*: urinary tract infection
NEC#: necrotising enterocolitis
+ve$ -positive

3.8 Clinical comparisons of ESBL and non-ESBL infections at the time of BSI

Comparisons made between children with and without ESBL-related KP BSIs at the time of the BSI showed that ESBL-KP BSI was more likely to manifest with pneumonia compared with non-ESBL-KP BSI, (p=0.04; 1.12 (1.02-1.21)). Children without an identifiable clinical focus were less likely to have ESBL-KP BSI (RR 0.88 [95%CI 0.80-0.96]; p=0.004). Although 284 (70.1%) children experienced fever, axillary

temperature >38^{0}C, this was not more likely to be associated with infection caused by ESBL-producing isolates (see Table 3.11). Other clinical events at the time of the blood culture were not significantly linked to ESBL-KP BSI.

Table 3.11: The clinical diagnosis at the time of *Klebsiella pneumoniae* (KP) bloodstream infection

Clinical diagnosis at the time of culture	ESBL-KP BSI n /N (%)	Non-ESBL KP BSI n /N (%)	p value	Risk ratio (95% CI)
Primary bloodstream infection with no identified source	159 /337 (47.2)	47/71 (66.2)	0.004**	0.88 (0.80-0.96)
Temperature >38^{0}C within 48 hours of obtaining the definitive blood specimen	238/334 (71.3)	46 /71 (64.8)	0.28	1.10 (0.91-1.32)
Pneumonia	108/336 (32.1)	14/71 (19.7)	0.04**	1.12 (1.02-1.21)
Vascular-catheter related	19/336 (5.6)	2/71 (2.8)	0.33	1.10 (0.95-1.28)
Peritonitis	16/336 (4.8)	4 /71 (5.6)	0.76	0.97 (0.77- 1.21)
UTI	16 /336 (4.7)	4 /71 (5.6)	0.76	0.97 (0.77-1.21)
Necrotising enterocolitis	18/338 (5.3)	2/71 (2.8)	0.37	1.09 (0.94-1.28)
Septic drip site (+ve culture of KP)	11/337 (3.2)	1 /71(1.4)	0.40	1.11 (0.93-1.33)
Soft tissue infection	9/336 (2.7)	1/71 (1.4)	0.53	1.09 (0.88-1.35)
Septic wound infection	13 /336 (3.8)	1/71 (1.4)	0.30	1.13 (0.97-1.32)

n/N stratum specific proportions
** denotes significance at p<0.05 level
ESBL-KP: extended-spectrum β-lactamase producing *Klebsiella pneumonia*
BSI: bloodstream infection
UTI: urinary tract infection
+ve: positive culture for KP from the septic drip site

3.9 Risk factors for ESBL infection

On univariate analysis, age < 12 months, moderate or severe underweight-for-age, HIV infection, resident in the PICU at the time of the KP BSI, cephalosporin exposure during the 12 month period prior to the development of KP BSI, mechanical ventilation prior to BSI, intravenous infusion for more than 3 days prior to BSI, indwelling urinary catheter prior to BSI, major surgery prior to BSI and a previous KP infection in the 12 month period before the KP-BSI were significant risk factors for ESBL infection.

On multivariate generalised linear modelling analysis, cephalosporin exposure during the 12 month period prior to the development of KP BSI, HIV infection, and intravenous infusion for more than 3 days prior to BSI remained significant risk factors for ESBL infection (see Table 3.12).

Table 3.12: Spectrum of factors associated with presumed ESBL-*Klebsiella pneumoniae* bloodstream infection (BSI)

Risk factors for ESBL-KP infection	Univariate analysis p-value; RR[$$] (95% CI)	Adjusted analysis p-value; aRR (95% CI)
Age (infant vs. >1y)	0.014; 1.15 (1.03-1.28)	0.09; 1.10(0.99-1.24)
Gender	0.17; 0.94 (0.86-1.03)	- -
Moderate or severe underweight for age	0.03; 1.11 (1.01-1.23)	0.23; 1.06 (0.96-1.16)
HIV-infection	<0.0001; 1.22 (1.13-1.30)	0.01; 1.12 (1.02-1.22)**
Patient in the PICU*	0.02; 1.11 (1.02-1.21)	0.36; 0.95 (0.84-1.06)
Inter-hospital transfer[$]	0.15; 1.05 (0.99-1.12)	- -
Cephalosporin exposure in the last 12 months pre-BSI	<0.0001; 1.30 (1.18-1.42)	<0.0001; 1.27 (1.15-1.40)**
Mechanical ventilation prior to BSI	<0.0001; 1.19 (1.10-1.29)	0.05; 1.16 (1.00-1.34)
IV fluids infusion>3days prior to BSI	<0.0001; 1.29 (1.14-1.45)	0.002; 1.21 (1.07-1.36)**
Indwelling urinary catheter prior to BSI	<0.0001; 1.20 (1.10-1.30)	0.32; 1.05 (0.95-1.16)
Major surgical procedure prior to BSI#	0.03; 1.11 (1.01-1.21)	0.85; 0.99 (0.88-1.11)
Excoriated skin	0.30; 1.05 (0.96-1.15)	- -
Previous KP infection within last 12m	0.01; 1.14 (1.03-1.26)	0.94; 1.01 (0.89-1.14)

[$$] RR risk ratio; *PICU: paediatric intensive care unit; # A major surgical procedure included a laparotomy (emergency or elective), a thoracotomy, a skin graft or a craniotomy, BSI, and bloodstream infection; $ Inter-hospital transfer: transferred from another hospital prior to the development of bloodstream infection; Multivariate model adjusted for HIV infection, age<12 months, underweight-for-age, mechanical ventilation, patient in the PICU at the time of the BSI, IV fluid infusion longer than 3 days prior to BSI, indwelling urinary catheter, major surgery prior to the BSI, Previous KP infection within last 12months, and cephalosporin exposure during last 12 months prior to BSI. aRR adjusted risk ratio. ** Factors with p-value <0.05 in the adjusted risk analysis;

3.10 Prior antibiotic exposure of hospitalised children with *Klebsiella pneumoniae* bloodstream infection

In view of the high prevalence of children with chronic health conditions, it was important to conduct an analysis exploring the influence of exposure to antibiotics on the type of KP infection. The analysis revealed that 40% (155) of 387 children had taken at least one of the following antibiotics in the 12 months preceding current hospitalisation: 2nd and/or 3rd and/or 4th generation cephalosporins, fluoroquinolones, macrolides, cotrimoxazole, aminoglycosides, piptazobactam, and carbapenems. Exposure to any of the stated antibiotics was significantly associated with having an ESBL-producing KP BSI [p-value <0.0001; RR 1.97 (95% CI 1.52-2.57)] compared to a non-ESBL associated infection on the univariate analysis.

On univariate analysis, significantly more children who received 2nd and/or 3rd and/or 4th generation cephalosporins, aminoglycosides, cotrimoxazole, carbapenams, and/or piptazobactam during the prior 12 months developed ESBL-KP BSI (see, table 3.13)

Table 3.13: Prior (up to 12 months) antibiotic exposure of hospitalised children with *Klebsiella pneumoniae* bloodstream infection (univariate analysis)

	ESBL-KP# n /N* (%)	Non-ESBL-KP n /N (%)	p value	Risk ratio (95% CI)
Number of children	319/388 (82.4)	69/388 (17.6%)	-	-
Exposure to a selection of antibiotics**	287/319(90)	31/68 (46.4)	<0.0001	1.97 (1.52-2.57)
2nd and/or 3rd and/or 4th generation Cephalosporins	190/205(92.8)	15 /69 (17.8)	<0.0001	1.31 (1.19-1.46)
Aminoglycosides	206/319 (64.6)	21/69 (30.4)	<0.0001	1.30 (1.16-1.44)
Cotrimoxazole	91/319 (28.5)	5/69 (7.3)	0.001	1.21 (1.12-1.31)
Carbapenems	65/319 (20.4)	4 /69 (5.8)	0.004	1.18 (1.10-1.28)
Piptazobactam	95/319 (29.8)	12/69 (17.4)	0.04	1.11 (1.02-1.22)
Fluoroquinolones	48/319 (15.1)	7/69 (10.1)	0.29	1.07(0.95-1.20)

ESBL-KP: extended-spectrum β-lactamases producing *Klebsiella pneumoniae*
* n/N: stratum specific proportions
** Exposure to the following antibiotics within the last 12 months: 2nd and/or 3rd and/or 4th generation cephalosporins, macrolides, fluoroquinolones, cotrimoxazole, aminoglycosides, piptazobactam, and carbapenems

3.11 Children presenting with diarrhoeal disease

Diarrhoeal disease is a seasonal condition. Traditionally, the season is associated with the highest number of children presenting to the institution with subsequent overcrowding of all the wards. Therefore, it was helpful to include an exploratory sub-group analysis for children presenting with diarrhoeal disease.

In the study, 137 children presented with diarrhoeal disease, which was acute in 114 and chronic in 23 children as one of their main admission complaints. In this group, 58 of 137 (77%) children were infants. The case-fatality rate of children with KP-BSI who presented with diarrhoeal diease: 44/137 (32%) died, 41 who manifested with acute diarrhoeal disease and 3 who had chronic diarrhoeal disease. Furthermore, 23/44 (52%) died within 3 days of the onset of BSI.

By univariate analysis, children presenting with diarrhoeal disease were more likely to be moderately or severely UWFA. Their characteristics are summarised in Table 3.14. They also had a shorter median time to the BSI after admission to hospital than those without diarrhoea; 5 versus 11 days, $p<0.0001$. Of 82 HIV-infected children, 52/82(63.4%) presented with diarrhoeal disease compared with 30/82 (36.6%) who did not present with diarrhoeal disease, p=<0.0001.

Table 3.14: Comparison of characteristics and outcome of children with KP BSI presenting with and without diarrhoeal disease (univariate analysis)

Variable	With diarrhoeal disease n (%)	Without diarrhoeal disease n (%)	p value	RR(95% CI)
N (all=410)	137 (33.4%)	273 (66.6%)	-	-
Gender(M:F)	66:71	146:127	0.31	0.87 (0.66-1.14)
Median age	5.2 (2.2-11.1)	5.1(1.2-29.4)	p = 0.48	-
Moderate or severe UWFA	96 (70.1)	145 (53.1)	0.001	1.32 (1.13-1.54)
HIV-infection	52 (38%)	30 (11%)	<0.0001	1.89 (1.47-2.44)
ESBL KPBSI	119 (86.9)	220 (80.6)	0.11	1.38 (0.91-2.12)
Median time to bacteraemia (IQR) days	5 (3-9)	11 (4-25)	p <0.0001	-
Median time to death days (IQR)	12 (5-25)	13 (6-32)	p = 0.11	-
Mortality	44 (32.1)	79 (28.9)	0.51	1.10 (0.82-1.51)

KPBSI: *Klebsiella pneumoniae* bloodstream infection
ESBL: extended-spectrum β-lactamase producing
UWFA: underweight-for-age
IQR: interquartile range

3.12 Laboratory changes at the time of the bloodstream infection

This section summarises haematological and pro-inflammatory results measured at the time of the KP BSI.

3.12.1 Haematological changes

Of the children, 71% (290/407) had normocytic normochromic anaemia at the time of the bacteraemic event. The mean haemoglobin was 9.7 g/dl SD ±2.18 and the

median mean corpuscular volume (MCV) was 84 (IQR 78-89) femtolitres. Children with ESBL-KP infection had a lower mean haemoglobin compared with those with non-ESBL infection, p=0.006. The differential WBC counts did not differ significantly between the two groups of children. The median platelet count, however, was significantly lower in the ESBL-infection group, p=0.003 (see Table 3.15). Anaemia was not more prevalent in children who were moderately or severely UWFA compared with those with normal weight-for-age z scores, 169/241 (71.6%) vs. 121/169 (71.6%); p=0.75; RR 0.98 (95% CI 0.81-1.16).

Elevated WBC were seen in 110 (33.7%) of 406 children with 223 (63.7%) of 350 children showing a band count of ≥10%. Of the children, 62 (15.3%) were pancytopaenic at the time of the KP BSI. The healthcare providers also saw thrombocytopaenia in the absence of pancytopaenia in 161 (39.3%) children.

Coagulation disturbances were evident in 73 (57.5%) of 127 children tested. More children, 66/108 (61.1%), with ESBL-KP infection had evidence of a coagulopathy compared with 7/19 (36.8%), with non-ESBL infection, p=0.048; RR 1.16(1.00-1.37).

Table 3.15: Comparisons of haematological indices in children with and without ESBL-KP bloodstream infection (N=410)

Variable	All children	n	ESBL (n=339)	n	Non-ESBL (n=71)	n	p value
Mean Hb (±sd) g/dl	9.71 (±2.18)	407	9.57 (±2.17)	338	10.5 (±2.13)	69	0.003
Median MCV (IQR)fl	84 (78-89)	407	84 (78-89)	338	85 (80-90)	69	0.18
Median WBC (IQR) $x10^9$ /l count	11.7 (6.24-20.09)	407	11.75 (6.14-19.86)	338	11.7 (6.9-22.07)	69	0.55
Median band count (IQR) $x10^9$/l	1.50 (0.43-4.16)	354	1.51 (0.38-4.12)	296	1.36 (0.64-5.03)	58	0.46
Percentage band count (IQR)	14 (5-27)	243	13 (5-27)	296	16 (8-28)	58	0.24
Neutrophil count (IQR) x 10^9/l	4.66 (1.48-10.32)	355	4.82 (1.26-10.31)	297	4.37 (2.26-10.15)	58	0.8
Lymphocyte count (IQR) $x10^9$/l	2.64 (1.27-4.37)	354	2.51 (1.21-4.31)	296	2.83 (1.53-4.55)	58	0.17
Median platelet count (IQR) $x10^9$/l	113(34-306)	405	98 (31-284)	337	183 (73-382)	68	0.003
Median C reactive protein (IQR) mg/l	108 (38-194)	203	108 (38-194)	174	111 (52-194)	29	0.8
Median procalcitonin (IQR) µg/l	15.25 (1.9-41.7)	106	13.3 (1.85-43.3)	96	24.65 (7.9-32)	10	0.34

Hb: haemoglobin; WBC: white blood cell; MCV: mean corpuscular volume; IQR: interquartile range; SD: standard deviation

There were reductions of one or more haematological cell lines in many children (see Table 3.16). Normocytic anaemia was the most common finding. Thrombocytopaenia was the second most common finding and was significantly more common in children with ESBL-KP infection, p=0.046; RR 1.0 (1-1.20) than in those with a non-ESBL infection. Leukopaenia was uninfluenced by whether the KP-infection was ESBL-related or not.

Anaemia with thrombocytopaenia was the most common bicytopaenia and was more common in children with ESBL-KP BSI, p=0.002; RR 1.78 (1.17-2.70). Leukopaenia with thrombocytopaenia was also more common in children with ESBL-KP infection, p=0.05; RR1.12 (1.02-1.22). Pancytopaenia was more common in children with ESBL-KP BSI, p=0.04; RR 1.13 (1.04-1.23) (see Table 3.16).

Table 3.16: Prevalence of haematological cell line reductions in hospitalised children with KP bloodstream infection

Cell line	ESBL n (%)	Non-ESBL n (%)	p value; RR (95% CI)
Anaemia (Hb<11)	246/339 (72.6%)	44 /71(62.0%)	0.07; 1.09 (0.98-1.22)
Leukopaenia	82/339 (24.3%)	14/71 (20.3%)	0.48; 1.04 (0.94-1.14)
Thrombocytopaenia	193/337 (57.3%)	30 /68(44.1)	0.046; 1.09(1-1.20)
Pancytopaenia	57/338(16.9%)	5/68 (7.4%)	0.05; 1.13 (1.04-1.23)
Thrombocytopaenia, no pancytopaenia	136/339 (40.1%)	25/71 (35.2%)	0.42; 1.03 (0.95-1.13)
Anaemia with thrombocytopaenia	153/339 (45.1%)	18/71 (25.4%)	0.002; 1.78 (1.17-2.70)
Anaemia with leukopaenia	71/339 (20.9%)	10 /71(14.1%)	0.19; 1.49 (0.81-2.74
Leukopaenia with thrombocytopaenia	66/339 (19.5%)	7/71(9.9%)	0.05; 1.12 (1.02-1.22)
Elevated band count>10%	186/294 (63.3)	37/56 (66.1%)	0.69; 0.96 (0.78-1.18)
Leucocytosis	110/337 (32.6)	27/69 (39.1)	0.3; 0.83 (0.60-1.16)

Hb: haemoglobin

3.12.2 Infective markers: C - reactive protein and procalcitonin values at the start of the KP BSI

In 287 children in whom the serum procalcitonin (PCT) or C-reactive protein (CRP) were determined, 263 (91.6%) had a raised PCT or CRP. This was uninfluenced by the ESBL status of the KP isolate (p=0.47) (see Table 3.17).

Table 3.17: C-reactive protein and procalcitonin concentrations at the time of KPBSI in children with *Klebsiella pneumoniae* Bloodstream infection

Variable	All children (n=410)	ESBL (n=339)	Non-ESBL (n=71)	p value
Median CRP (IQR) mg/l	(n=203) 108 (38-194)	(n=174) 108 (38-194)	(n=29) 111 (52-194)	0.8
n/N (%) with CRP >10mg/l	186/203 (91.6)	160/174 (92)	26/29 (89.7)	0.68
n/N (%) with CRP >80mg/l	120/203 (59.1)	103/174 (59.2)	17 /29 (58.6)	0.95
Median PCT (IQR) ng/ml	(n=106) 15.25 (1.9-41.7)	(n=96) 13.3 (1.85-43.3)	(n=10) 24.65 (7.9-32)	0.34
n/N (%) with PCT >0.5ng/ml	95/106 (89.6)	85/96 (88.5)	10/10 (100)	0.26
n/N (%) with PCT >2ng/ml	79/106 (74.5)	69/96 (71.9)	10/10 (100)	0.05

n= number of cases
n/N: stratum specific proportions
ESBL: extended-spectrum β-lactamase producing
CRP: C-reactive protein
PCT: procalcitonin
IQR: interquartile range

CHAPTER FOUR: ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *KLEBSIELLA PNEUMONIAE* ISOLATES AND ANTIBIOTIC THERAPY

4.1 Antibiotic sensitivity patterns of the isolates overall

All isolates, both ESBL- (339/410) and non-ESBL-producing (71/410), were resistant to ampicillin. Of these, 21% (86/410) were sensitive to gentamicin; in contrast, 76.1% (312) were sensitive to amikacin. Out of 349 tested, 211 (60.5%) were sensitive to piptazobactam; 117/402 (29.1%) isolates were sensitive to coamoxy-clavulinic acid. All isolates were sensitive to the carbapenems imipenem and meropenem, and 402/404 (99.5%) isolates were reported sensitive to ertapenem.

4.2 Antibiotic therapy

Data on the antibiotic therapy that was instituted in response to the BSI were available in 398 children. Antibiotic therapy was considered appropriate if the NHLS laboratory reported the organism sensitive to the specific antibiotic. The empiric antibiotic prescribed in response to the BSI was appropriate in 307/398 (77.1%) children: 241/398 (73.5%) children with ESBL KP BSI and 66/70 (94.3%) children with non-ESBL KP BSI. Once laboratory results became available, healthcare providers adjusted the antibiotic cover; this delay did not reflect an increase in crude mortality p=0.29, RR 0.83 (95% CI: 0.59-1.16), there were 87/307(28.3%) children on appropriate empiric antibiotics who died versus 31/91 (34.1%) children on inappropriate empiric antibiotics. Table 4.1 summarises the susceptibility of KP isolates to antibiotics or antibiotic combinations that are frequently used in clinical practice at RCWMCH.

Table 4.1: Susceptibility of *Klebsiella pneumoniae* isolates to commonly utilised antibiotics or antibiotic combinations over the 6 year study period 2006-2011

	Ampicillin + Gentamicin		Cefotaxime		Ampicillin + Amikacin		Piptazobactam + Amikacin		Ceftriaxone + Amikacin		Co-Amoxyclav		Ertapenem	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Community-acquired BSI	15/21	(71.4)	14/21	(66.7)	19/21	(90.5)	20/21	(95.2)	19/21	(90.5)	18/21	(85.7)	19/19	(100)
Nosocomial BSI	60/361	(16.6)	48/362	(13.3)	273/362	(75.4)	299/345	(86.7)	273/362	(75.4)	90/354	(25.4)	356/358	(99.4)
HCA BSI	11/27	(40.7)	9/27	(33.3)	20/27	(74.1)	23/26	(88.5)	20/27	(74.1)	9/27	(33.3)	27/27	(100)
Totals	86/409	(21.0)	71/410	(17.3)	312/410	(76.1)	342/392	(87.2)	312/410	(76.1)	117/402	(29.1)	402/404	(99.5)

n/N: stratum specific proportions
BSI: Bloodstream infection
HCA: Healthcare-associated infection

An initial empiric combination of ampicillin plus amikacin would have been effective in 90.5% (19/21) of community-acquired KPBSI whereas the combination of ampicillin plus gentamicin would have been appropriate in 71.3% (15/21). From the susceptibility patterns, ertapenem would have provided adequate cover to 356/358 (99.4%) of nosocomially acquired KPBSI.

4.2.1 Sub-analysis on antibiotic susceptibility patterns over two periods 2006-2008 and 2009-2011

Antibiotic susecptibility patterns for KP isolates from 2006 to 2008 and 2009 to 2011 are summarised in Tables 4.2 and 4.3 respectively.

- In the first period 2006-2008 ertapenem would have provided 100% (187/187 isolates susceptible), cover for all categories of KP BSI as empiric cover; however in the second period 2009-2011, cover was incomplete for suspected serious nosocomial KP BSI at 98.9% (187/189 isolates susceptible) but complete for community acquired infection (CAI) [(10/10 isolates susceptible)] and complete for healthcare-associated KPBSI (9/9 isolates susceptible).
- Given the number of isolates, in the period 2006-2008 ampicillin plus gentamicin combination would have covered 6/11 (54.6%) isolates compared to a higher proportion 9/10 (90%) isolates for CAI in the period 2009-2011.
- Regarding the combination of ampicillin plus amikacin as empiric broad-spectrum treatment of suspected community acquired infections, in the periods 2006-2008 and 2009-2011, 10/11 (90.9%) of isolates and 9/10 (90%) isolates respectively were susceptible to this combination
- The cover provided by the combination of ceftriaxone plus amikacin for nosocomial KP BSI declined markedly over the two periods 2006-2008 and 2009-

2011 from 87.9% (152/173 isolates susceptible) cover to 64% (121/189 isolates susceptible) isolate cover.

- Over the periods, 2006-2008 and 2009-2011, the cover provided by the combination of piptazobactam plus amikacin for nosocomial BSI diminished from 91.8 %(157/171 isolates susceptible) to 81.6% (142/174 isolates susceptible).
- There was a shift in cefotaxime sensitivity from 45.5% (5/11) in the first period 2006-2008 to 66.7% (9/10) in the second period 2009-2011.
- Cover by co-amoxyclav for CAI over the two periods was higher at 81.9% (9/11 isolates susceptible) and 90% (9/10 isolates susceptible) for CAI compared to 28% (47/168 isolates susceptible) and 23.1% (43/182 isolates susceptible) for nosocomial isolate cover
- Overall, the sensitivity patterns of KP isolates from children who had HCA BSI was very similar to those with nosocomial BSI (with respect to ampicillin plus amikacin; piptazobactam plus amikacin; ceftriaxone plus amikacin; and ertapenem), or was positioned between nosocomial and community BSI, (with respect to cover by ampicillin plus gentamicin; cefotaxime and co-amoxyclav).

Table 4.2: Susceptibility of *Klebsiella pneumoniae* isolates to commonly utilised antibiotics or antibiotic combinations for the period 2006-2008

	Ampicillin + Gentamicin		Cefotaxime		Ampicillin + Amikacin		Piptazobactam + Amikacin		Ceftriaxone + Amikacin		Co-Amoxyclav		Ertapenem	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Community-acquired BSI	6/11	(54.6)	5/11	(45.5)	10/11	(90.9)	10/11	(90.9)	10/11	(90.9)	9/11	(81.8)	9/9	(100)
Nosocomial BSI	33/172	(19.2)	29/173	(16.8)	152/173	(87.9)	157/171	(91.8)	152/173	(87.9)	47/168	(28.0)	169/169	(100)
HCA BSI	4/9	(44.4)	3/9	(33.3)	9/9	(100)	9/9	(100)	9/9	(100)	3/9	(33.3)	9/9	(100)
Totals	43/192	(22.4)	37/193	(19.2)	171/193	(88.6)	176/191	(92.2)	171/193	(88.6)	59/188	(31.4)	187/187	(100)

n/N: stratum specific proportions
BSI: Bloodstream infection
HCA: Healthcare-associated infection

Table 4.3: Susceptibility of *Klebsiella pneumoniae* isolates to commonly utilised antibiotics or antibiotic combinations for the period 2009-2011

	Ampicillin + Gentamicin		Cefotaxime		Ampicillin + Amikacin		Piptazobactam + Amikacin		Ceftriaxone + Amikacin		Co-Amoxyclav		Ertapenem	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Community-acquired BSI	9/10	(90.0)	9/10	(90.0)	9/10	(90.0)	10/10	(100)	9/10	(90.0)	9/10	(90.0)	10/10	(100)
Nosocomial BSI	27/189	(14.3)	19/189	(10.1)	121/189	(64.0)	142/174	(81.6)	121/189	(64)	43/186	(23.1)	187/189	(98.9)
HCA BSI	7/18	(38.9)	6/18	(33.3)	11/18	(61.1)	14/17	(82.4)	11/18	(61.1)	6/18	(33.3)	18/18	(100)
Totals	43/217	(19.8)	34/217	(15.7)	141/217	(65)	166/201	82.3	141/217	(65)	58/214	27.1	215/217	(99.1)

n/N: stratum specific proportions
BSI: Bloodstream infection
HCA: Healthcare-associated infection

4.2.2 Profile of the non-ESBL isolates antibiotic sensitivity prevalence

Of the 410 KP isolates, 71 (17.3%) were non-ESBL producers. They were all sensitive to cefotaxime, ceftriaxone (3rd generation cephalosporins) and cefepime (4th generation cephalosporin), amikacin, ciprofloxacin, and the carbapenems ertapenem, imipenem, and meropenem. The isolates were also sensitive to gentamicin, piptazobactam, and coamoxyclav in 94.4%, 91.6%, and 93.0% of cases respectively. Of the isolates, 53 of 71 (74.7%) were sensitive to cotrimoxazole. The combination of ceftriaxone plus amikacin would have provided adequate cover for all the children with non-ESBL KPBSI but would not have provided appropriate antibiotic cover for 98 (28.9%) children with ESBL BSI.

4.2.3 Profile of the ESBL isolates resistance patterns

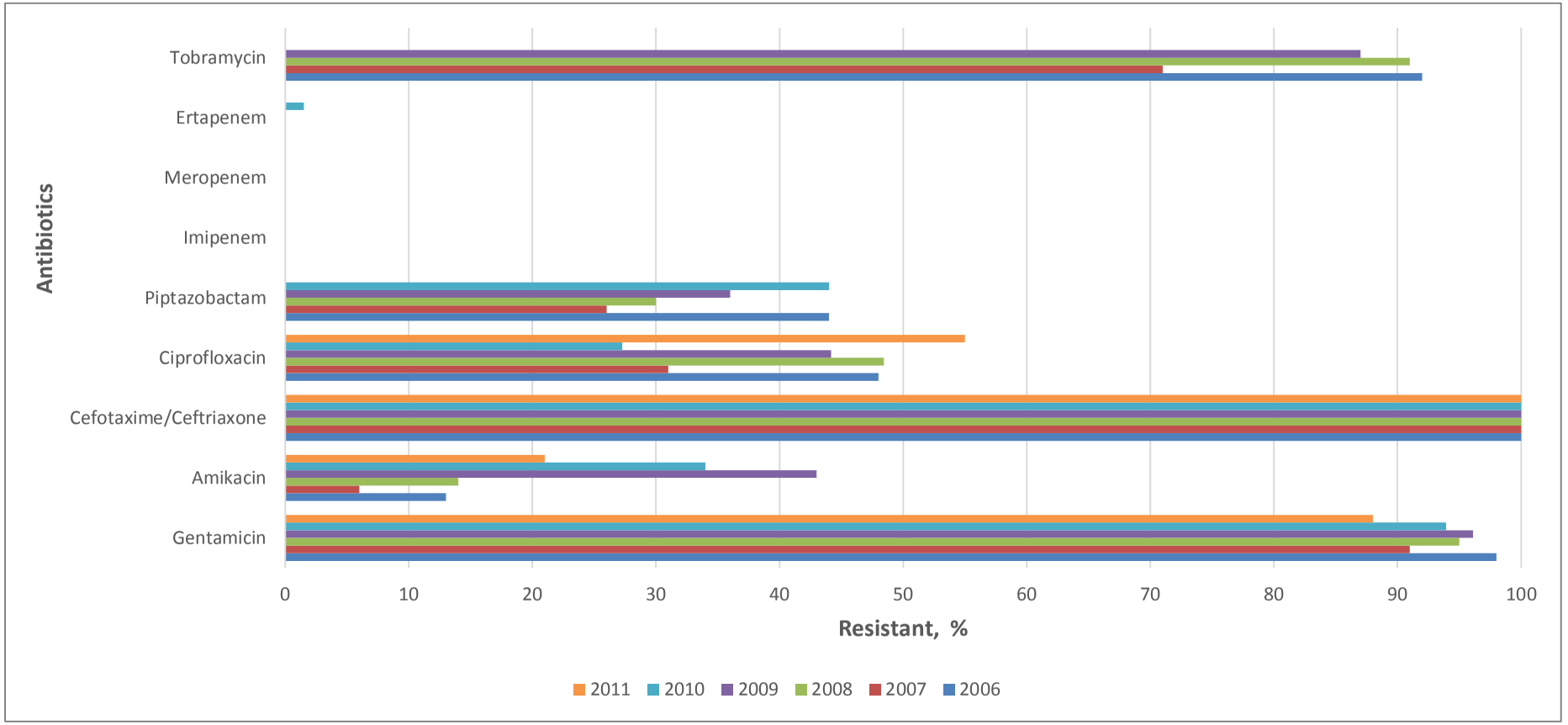
All isolates were resistant to ampicillin and cefotaxime, ceftriaxone and cefepime. Resistance to cotrimoxazole was reported in 313/339 (92.3%) isolates. Out of 339 tested, 51 (15.0%) were sensitive to coamoxyclav; 241/339 (71.1%) of the isolates were sensitive to amikacin, and 146/282 tested (51.8%) were sensitive to piptazobactam. At the same time, 197/339 (58.1%) children had ciprofloxacin-sensitive isolates. Additionally, the combination empiric therapy of piptazobactam plus amikacin would not have provided appropriate antibiotic cover to 50 (15.6%) children who had ESBL-KP BSI. The same empiric combination therapy would not have provided adequate cover for 9/33 (27.3%) children living in intermediate-care facilities. Bearing in mind that 96.3% (n=79) of the HIV-infected children had an ESBL infection, 26 (31.7%) would not have received appropriate cover if healthcare providers chose an antibiotic regimen consisting of ceftriaxone plus amikacin. All

isolates were sensitive to the carbapenems: imipenem and meropenem. Two were resistant to ertapenem (see Figure 4.3).

4.2.4 ESBL and non-ESBL isolates from HIV-infected children

Of the 82 HIV-infected children, 62 (75.6%) were on cotrimoxazole prophylaxis prior to the KP BSI. Of these infected children, 79 (96.3%) had ESBL-producing KP isolates compared to 3 (3.7%) who had non-ESBL KPBSI (Fisher's exact p =0.001). The ESBL-KP isolate was resistant to cotrimoxazole in 78 (98.7%) of HIV-infected children.

Figure 4.3: Proportion of ESBL-KP isolates demonstrating intermediate or highly resistant antibiotic profiles over the study period



ESBL-KP: extended-spectrum beta-lactamase producing *Klebsiella pneumonia*

4.3 Polymicrobial blood results

Of the study children, 69/410 (16.8%) had more than one bacterial pathogen isolated in the same blood culture as the one identifying the KP. These organisms included (see Table 4.4):

Table 4.4: Clinical features associated with polymicrobial blood culture results in 69 children

Other organism in the blood culture	n	(%)	Clinical features at the time of the blood culture
Escherichia coli	20	29.0	8 Pneumonia,6 no focus,3 UTI, 2 NEC,1septic wound
Enterococcus faecalis	11	15.9	5 Pneumonia, 4 no focus, 1 endocarditis, 1 CV-line-related
ESBL KP(other)	8	11.6	5 Pneumonia, 1 no focus, 1UTI, 1 CV-line related
Acinetobacter baumannii	7	10.1	5 No focus, 1 pneumonia, 1 septic wound
Serratia species	6	8.7	4 No focus, 1 pneumonia, 1 bowel obstruction
Enterobacter cloacae	4	5.8	2 No focus, 1 pneumonia, 1 NEC
Staphylococcus aureus(SA)	3	4.4	2 No focus, 1 pneumonia
Enterococcus faecium	2	2.9	2 Pneumonia
Methicillin resistant SA	1	1.5	Pneumonia
Streptococcus pneumoniae	1	1.5	Pneumonia
KP oxytoca	1	1.5	Peritonitis
Pseudomonas aeruginosa	1	1.5	No focus
Citrobacter freundi	1	1.5	Peritonitis
Candida species	1	1.5	Peritonitis
Streptococcus mitis	1	1.5	No focus
Staphylococcus epidermidis	1	1.5	Pneumonia
Total	**69**	**100%**	

UTI: urinary tract infection
NEC: necrotising enterocolitis
CV-line: central venous line

There were 30 female (43.5%) and 39 (56.5%) males. Their median age was 4.5 (IQR 1.1-10.8) months, 52 (75.4%) were less than 12 months. Of the children, 12 (17.4%) were HIV-infected and 23 (33.3%) were untested (see Table 4.5).

There was a concomitant underlying condition in 44 (63.8%) of the children. Four (5.8%) children had community acquired KPBSI; the other 65 (94.2%) had nosocomial infection or HAI; of these infections 53 (81.5%) were caused by ESBL-producing isolates. The children did not appear to have any distinguishing clinical features; 52 had a temperature> 38°C at the time of the bacteraemic event.

Children with polymicrobial BSI (54/69; 78.3%)were not more likely to have an ESBL-KP infection compared with children with single organism KP BSI (285/341; 83.6%); p=0.29; RR 0.94 (95%CI: 0.82-1.07) Apart from an older median age of the group with non-ESBL-KP infection, 21.5 (IQR 12.6-67.2) versus 3.1 (IQR 1.1-7.6) months, p value <0.001, there were no significant differences on univariate analysis between children with polymicrobial and single-organisms KP BSI (see Table 4.5 and 4.6). Additionally, they were not at greater risk of dying: 25/69 (36.2%) versus 98/341 (28.7%) with p= 0.22, RR 1.26 (0.88-1.80) on univariate analysis.

Table 4.5: Characteristics of 69 children with polymicrobial (including *Klebsiella pneumoniae*) bloodstream infection

Variable	Number		ESBL KPBSI		non-ESBL KPBSI		Univariate RR	
	n	(%)	n	(%)	n	(%)	pvalue	RR(95% CI)
Number of children (%)	n=69	(100)	n=54	(78.3)	n=15	(21.7)	0.003	0.94(0.82-1.07)
Median[IQR] age in months	4.50	(1.1-10.8)	3.1	(1.1-7.6)	21.5	(12.6-67.2)	<0.001	-
HIV negative	34	(49.3)	26	(48.1)	8	(53.3)	0.72	0.97(0.75-1.23)
HIV-infected	12	(17.4)	12	(22.2)	0	-	0.05	1.36(1.16-1.58)
Unknown HIV status	23	(33.3)	16	(29.6)	7	(46.7)	0.21	084(0.62-1.14)
Moderate-Severe UWFA	43	(62.3)	34	(63.0)	9	(60.0)	0.83	1.03(0.79-1.33)
Community-acquired BSI	4	(5.8)	1	(2.0)	3	(20.0)	-	-
Nosocomial BSI	57	(82.6)	48	(88.9)	9	(60.0)	-	-
HCA BSI	8	(11.6)	5	(9.3)	3	(20.0)	-	-

IQR: interquartile range
UWFA: underweight-for-age
ESBL: extended-spectrum β-lactamase
BSI: bloodstream infection

Table 4.6: Comparison of characteristics of children with polymicrobial and single-pathogen *Klebsiella pneumoniae,* bloodstream infection (KP BSI)

Factor	Polymicrobial	Single-pathogen	Univariate analysis	
	n/N (%)	n/N (%)	RR (95% CI)	p-value
Age (infant vs. >1y)	52/69 (75.4)	230/341 (67.5)	1.39 (0.84-2.30)	0.20
Gender(Male:female)	39:30	173:168	1.21 (0.79-1.88)	0.38
Moderate and severe underweight for age	43/69 (62.3)	198/341 (58.1)	1.16 (0.74-1.81)	0.51
ESBL-KP BSI	54/69 (78.3)	285/341 (83.6)	0.75 (0.45-1.26)	0.28
HIV-infection	12/46 (26.1)	70/242 (28.9)	0.84 (0.47-1.49)	0.56
Anaemia	46/69 (66.7)	244/341 (71.6)	0.83 (0.53-1.30)	0.43
Patient in the PICU	12/69 (17.4)	91/341 (26.7)	0.63(0.35-1.12)	0.12
Concomitant chronic medical condition	44/69 (63.8)	219/341 (64.2)	0.98 (0.63-1.54)	0.94
Pre-hospitalisation antibiotic exposure	22/67 (32.8)	133/341 (41.6)	0.74 (0.46-1.18)	0.20
Mechanical ventilation prior to BSI	21/69 (30.4)	134/341 (39.6)	0.72 (0.45-1.16)	0.18
IV fluids infusion>3days prior to BSI	45/69 (65.2)	232/341 (68.0)	0.82 (0.53-1.26)	0.37
Indwelling urinary catheter prior to BSI	18/68 (26.5)	133/341 (39.6)	0.61 (0.37-1.00)	0.05
Major surgical procedure prior to BSI*	19/69 (27.5)	112/341 (33.1)	0.80 (0.49-1.30)	0.37
Excoriated skin	20/68 (29.0)	110/341 (32.5)	0.89 (0.55-1.43)	0.63
Previous KP infection within last 12m	5/69 (7.2)	37/341 (10.9)	0.68 (0.29-1.60)	0.38

*A major surgical procedure included a laparotomy (emergency or elective), a thoracotomy, a skin graft or a craniotomy.
RR: risk ratio; n/N: stratum specific proportions
PICU: paediatric intensive care unit
ESBL-KP: extended-spectrum β-lactamase producing *Klebsiella pneumoniae*
IV: intravenous
BSI: bloodstream infection

CHAPTER FIVE: OUTCOME OF CHILDREN WITH *KLEBSIELLA PNEUMONIAE* BLOODSTREAM INFECTION

5.1 Clinical complications associated with *Klebsiella pneumoniae* bloodstream infection

Children with KPBSI experienced a number of organ dysfunction complications that affected their outcomes. The following sub-sections summarised respiratory, renal, hepatic, and haematological complications. As these complications were on the causal pathway to death because of the BSI, they were not analysed as independent risk factors in the multivariate model to avoid the problem of co-linearity.

5.1.1 Respiratory failure in children with and without ESBL-KP bloodstream infection

Of the children, 144 (35.2%) required mechanical ventilatory support at the time of the KPBSI. Children with ESBL KPBSI were more likely to develop respiratory failure than those with non-ESBL KPBSI, 131/339 (38.6%) versus 13/71 (18.3%), risk ratio 2.11 [95% CI: 1.27 – 3.51] (see Table 5.1).

5.1.2 Renal function changes in children with and without ESBL-KP bloodstream infection

Renal dysfunction occurred in 105/410 (25.6%) children if the serum creatinine was above the upper limit for age. This was not influenced by whether the KP BSI was ESBL-related in 86/339 (25.4%) children or not [19/71 (26.8%) children], p-value= 0.81: RR 0.95 (95% CI 0.62-1.45) (see Table 5.1).

5.1.3 Liver function dysfunction in children with and without ESBL-KP bloodstream infection

Hepatic dysfunction, as evidenced by elevated transaminases, occurred in 71/410 (17.3%) of the children. There was no significant association with ESBL infection, 62/339(18.3%) children had hepatic dysfunction compared to 9/71(12.7%) children with non-ESBL BSI, p=0.26: RR 1.44(0.75-2.77) (see Table 5.1).

5.1.4 Coagulation disturbances in children with and without ESBL-KP bloodstream infection

Healthcare providers performed coagulation studies on 127 children. Coagulopathy was not significantly more common in 66/108 (61.1%) children with ESBL infection compared to those 7/19 (36.8%) children with non-ESBL infection, p=0.05: RR 1.66(95%CI 0.90-3.05). Table 5.1 summarises the various organ dysfunctions in relation to ESBL-infection.

Table 5.1: Prevalence of organ dysfunction in children with ESBL-KP bloodstream infection

Variable (by ESBL)	All children n/N(%)	ESBL(339) n (%)	Non-ESBL(71) n (%)	p value; RR (95% CI)
Respiratory failure	144/410(35.2)	131/339(38.8)	13/71 (18.3)	0.001; 2.11 (1.27-3.51)
Renal dysfunction	105/410 (25.6)	86/339 (25.4)	19/71 (26.8)	0.81; 0.95 (0.62-1.45)
Hepatic dysfunction	71/410 (17.3)	62/339(18.3)	9/71(12.7)	0.26; 1.44 (0.75-2.77)
Coagulopathy	73/127 (57.5)	66/108 (61.1)	7/19 (36.8)	0.05; 1.66 (0.90-3.05)

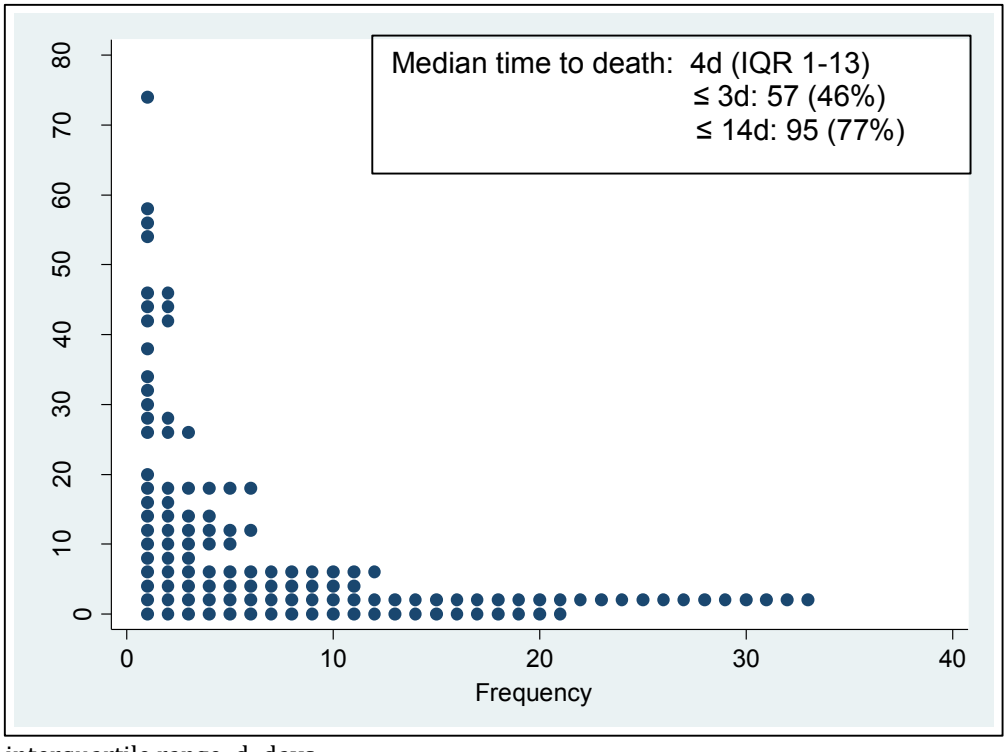
RR - risk ratio
ESBL BSI-
n/N stratum specific proportions

5.2 Mortality

Over the 6-year period, 123 of the study children died, giving a crude case fatality rate of 30% (123/410). The median time to death was 4 days (IQR 1-13 days). There were 95/123 (77%) deaths occurring within 14 days of positive KP blood culture, and 57 (46%) children died within 3 days of the positive KP-blood culture (see Figure 5.1 and Table 5.2).

Of the study children, 21 died (i.e. 17.1% of all deaths) on the day the blood culture were taken, including two who died on the day of admission to hospital. Community acquired-KP infection caused one of these deaths, and the other 20 children had nosocomial KP infections. ESBL-producing KP caused 19/ 20 infections. Six untreated children died before healthcare providers could initiate appropriate antibiotic therapy.

Figure 5.1: Time to death in 123 children with *Klebsiella pneumoniae* bloodstream infection



IQR: interquartile range; d: days

Of 123 deaths, 111/339 (32.7%) were ESBL-related compared to 12/71(16.9%) that were non-ESBL-related, p=0.008, RR 1.94 (95% CI: 1.13-3.32). Septicaemia was the documented cause of death in 90/123 (73.2%) of cases, and pneumonia accounted for 13.8% of the mortalities (see Table 5.2). There were 4/21(19.1%) children that died in whom the KPBSI was community acquired compared to 119/389 (30.6%) deaths in children with healthcare-related KP BSI, p=0.26, RR 1.61 (95% CI: 0.66-3.93).

Table 5.2: Time to death in 3 time categories in study children with *Klebsiella pneumoniae* bloodstream infection

	Time to death from KP BSI			
Cause of death	≤ 3 days	3.1-14 days	>14 days	Totals
	n %	n %	n %	n %
Septicaemia	48 (39)	28 (22.8)	14 (11.4)	90 (73.2)
Pneumonia	6 (4.9)	4 (3.3)	7 (5.7)	17 (13.8)
Other	3 (2.4)	6 (4.9)	7 (5.7)	16 (13.0)
Totals	57 (46.3)	38 (31)	28 (22.8)	123 (100)

Death from septiceamia was most likely to occur within 14 days of the KP BSI, 76/90(84%) children died compared to non-septicaemia where 19/33 (58%) died during the same period; p= 0.002.

Table 5.3: Characteristics of children with KP-bloodstream infection who lived or died during the 6-year study period (2006-2011)

Characteristic	Died n/N (%)	Alive n/N (%)	p value
Number of children (%)	123/410 (30)	287/410 (70)	-
Median (IQR) age in months	4.4 (1.3-14.9)	5.5 (1.9-16.2)	0.33
Gender: male: female	61:62	151:136	0.58
Age <1year (n, %)	88 (71.5)	194 (67.6)	0.43
Moderate to severely underweight-for-age	78 (63.4)	163 (56.8)	0.21
HIV-infection	39 (32)	43 (15.0)	0.001
Unknown HIV status	29 (23.6)	93 (32.4)	-
Chronic underlying medical condition	87 (70.7)	176/287 (61.3)	0.07
Median time to bloodstream infection from admission (IQR) in days	8 (4-19)	7 (3-20)	0.48
Median time to death from bloodstream infection (IQR) in days	4 (1-13)	-	-
Hospitalisation within previous 28 days	58 (47.1)	113/283 (39.9)	0.18
Bloodstream infection without clinical focus	47 (38.5)	159/286 (55.6)	0.002

n/N: stratum specific proportions; IQR: interquartile range

Of all the children who died, 29% (36/123) had no chronic underlying medical disorder. At the same time, 32% (39/123) of the children were HIV-infected (see Tables 5.3 and 5.4). Chronic gastrointestinal and congenital cardiac conditions were present in 10% (13/123) and 12% (15/123) of children respectively.

Table 5.4: The chronic medical condition in children that died with *Klebsiella pneumoniae* bloodstream infection

Chronic underlying medical condition in 123 children that died	n	%
Nil	36	29.3
HIV infection	39	31.7
Congenital heart disease	15	12.2
Gastrointestinal	13	10.6
Neoplasm	5	4.1
Neurology	4	3.3
Renal	3	2.4
Aplastic anaemia	3	2.4
Tuberculosis	2	1.6
Metabolic	1	0.8
Congenital CMV*	1	0.8

* Cytomegalovirus infection

5.3 The role of HIV infection in outcome

Mortality was higher in the HIV-infected group of children compared with those who were uninfected. Of the 82/288 (28.5%) children known to be HIV-infected, 39 /82 (47.5%) died compared to 55/ 206 (26.7%) HIV-uninfected children, RR 1.78 (1.29-2.45), p=0.001 (see Figure 5.3). Table 5.5 summarises the outcome of HIV-infected children on ART over the study period.

The proportion of HIV-infected children peaked at 26.7% (23/86) in 2009 with the peak of KP BSI cases (86/410) and then reduced to 7.8% (4/51) in 2011. Of the HIV-infected group, 41/82 (50 %) children were on antiretroviral therapy (ART) at the

time of the KP BSI. In 2006 71.4% (10) of 14 HIV-infected children died, at that time 14.3% of the 14 children were on ART; mortality in the HIV-infected children with KPBSI was high throughout the 6 year study period ranging from 35.3% to 71.4% despite the overall trend of improved access to ART. Even when all HIV-infected children (4) were on ART, mortality from KP SBI was 50% in 2011.

Table 5.5: Number and percentages of HIV-infected children on ART who lived or died over the study period

Variable	2006	2007	2008	2009	2010	2011
Number of children with KP BSI	70	50	73	86	80	51
Number of HIV-infected children	14	11	17	23	13	4
% with HIV infection of total KP BSI	20	22	23.3	26.7	16.3	7.8
% HIV-infected children on ART	14.3	45.5	64.7	47.8	61.5	100
N of HIV-infected children dying per year	10	4	6	12	5	2
% of all HIV-infected children that died	71.4	36.4	35.3	52.2	38.5	50

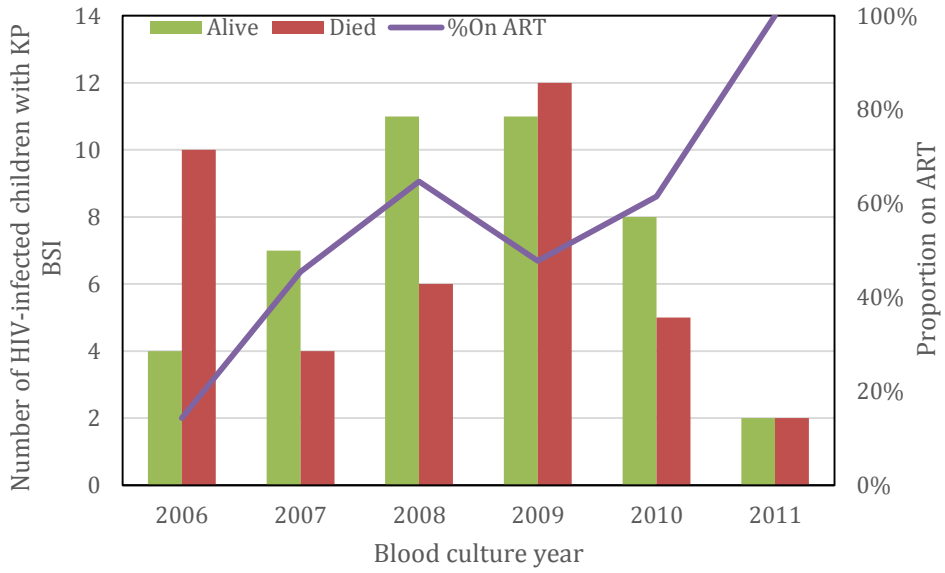
KP BSI: *Klebsiella pneumoniae* bloodstream infection
ART: antiretroviral therapy
%: percentage

The median time on ART prior to the BSI was 19 IQR (8-66) days in the 41 children on ART. Five (12.2%) of these children had been on ART for 6 months or longer at the time of the KPBSI. Within the HIV-infected group the risk of death was reduced in those on ART (14/39 (35.9%) compared to those 25/39 (64.1%) children not on ART; p=0.02, RR 0.56(CI 0.34-0.92) (see Figure 5.3). There was 1/ 5 (20%) child who died who had been on ART for 6 months or longer compared to 13/36 (36.1%) children who died and had been on ART for less than 6 months, p= 0.44.

The analysis did not show significant links between severe immunosuppression-for-age and mortality, 21/39 (53.9%) children with severely immunosuppressed CD4 for age who died compared to 21/43(48.8%) children who were severly immunosuppressed and lived, (p=0.65; RR 1.10 (0.70-1.75). The same was true for

those 26/39 (66.7%) children with severely immunosuppressed absolute CD4 count for age who died compared to 29/43 (67.4%) children who lived, p=0.94; RR 0.98 (0.61-1.59). However, the timing of the CD4 lymphocyte testing was not uniform in that the healthcare providers collated results within the preceding 3-month period of the BSI. In the presence of HIV disease, mortality was not significantly influenced by moderate or severe underweight-for-age status, 28/39 (71.8%) children died compared to 33/43 (76.7%) children who lived, (p= 0.61; RR 0.88 (CI 0.54-1.43).

Figure 5.3: The number of HIV-infected children with KPBSI and the proportion on ART over the study period 2006-2011



KP BSI: *Klebsiella pneumoniae* bloodstream infection
ART: antiretroviral therapy

5.4 The cause of death

Healthcare providers recorded the cause of death for 122 children. In one child admitted with severe burn injury, the death was unexpected and occurred in the burn ward soon after transfer from the PICU (see Table 5.6).

Table 5.6: Cause of death in 123 children with *Klebsiella pneumoniae* bloodstream infection

Cause of death	n	Percentage
Septicaemia	90	73.2
Pneumonia	17	13.8
Multi-organ dysfunction syndrome	4	3.3
NEC	3	2.4
Neoplasm	2	1.6
Hepatitis	1	0.8
Peritonitis	1	0.8
Volvulus	1	0.8
Tyrosinaemia, liver failure	1	0.8
Congestive cardiac failure	1	0.8
Bacterial endocarditis	1	0.8
Unknown(not recorded, burns patient)	1	0.8

KP: *Klebsiella pneumoniae*
NEC: necrotising enterocolitis

5.5 Post-mortem findings

Of the 123 study children that died, 15 had post mortem examinations. Table 5.7 summarises the main necropsy findings. Necrotising tissue breakdown was a common post mortem finding.

Table 5.7: Key post mortem features in 15/123 hospitalised children with *Klebsiella pneumoniae* bloodstream infection

Post mortem feature	n	Percentage (%)
Multi-lobar necrotising pneumonia	15	100
Disseminated intravascular coagulopathy	8	53.3
Skin excoriation and ulceration	8	53.3
Acute adrenal haemorrhage	7	46.7
Acute tubular necrosis	6	40
Abscess formation	5	33.3
Necrotising enterocolitis	5	33.3
Necrotising oesophagitis	4	26.7
Septic pulmonary emboli	4	26.7
Peritonitis with abundant pus	2	13.3

The Tables 5.8 and 5.9 describes characteristics of 15 children who died and their post mortem findings. All 15 children had evidence of bronchopneumonia at post mortem. Thymic involution was evident in 14 of the 15 children. All 15 children died within 6 days of developing KP BSI.

Acute tubular necrosis (ATN), disseminated intravascular coagulopathy (DIC), and adrenal haemorrhage were prominent additional features. A common finding was necrotising inflammation, indicative of tissue destruction. KP was positively identified in 8/15 lung specimens but was not mentioned in the post-mortem reports of the other seven children. In one in these seven children, disseminated CMV infection was present. This child was HIV-uninfected. Another child, with KP confirmed at post-mortem, also had evidence of *Pneumocystis jirovecii* (PJP) and measles pneumonia; this child's HIV status was unknown.

Table 5.8: Characteristics of 15 children with *Klebsiella pneumoniae* bloodstream infection (KPBSI) who had post-mortem examinations

Case	Sex	Age (m)	ESBL-KP	Underlying medical condition	Admission diagnosis	KPBSI Event diagnosis	HIV-status	Time to BSI from admiss-ion(d)	Time to death from BSI (d)
1	M	0.2	0	Infant of diabetic mother, PDA,VSD	Hydronephrosis ascites	Septic shock	UK	4	0
2	M	72	1	Nil	Stevens Johnson Syndrome	Septic shock	UK	20	0
3	M	3	1	Nil	Gastroenteritis	Septic shock	NEG	3	0
4	F	22	0	Caustic soda oesophageal damage	Vomiting	Pneumonia	UK	0	5
5	F	49	1	Dermato-myositis	Septic skin lesions and cough	Pneumonia	NEG	18	6
6	F	7	1	Short bowel syndrome	Pneumonia	Pneumonia	NEG	177	5

7	M	3	1	Congenital cardiac-ASD,PDA	Pneumonia	Pneumonia	UK	6	1
8	M	0.8	1	Nil	Gastroenteritis	septic shock, NEC	Exposed	14	6
9	F	4	1	Cystic fibrosis	Acute severe malnutrition	Pneumonia	NEG	0	2
10	F	3	1	Nil	Pneumonia	Pneumonia	UK	3	0
11	F	1	1	HIV-disease	Pneumonia	Pneumonia	POS	4	0
12	F	8	1	Nil	Gastroenteritis, pneumonia	Pneumonia	NEG	8	5
13	M	1	1	HIV-disease	Gastroenteritis, pneumonia	Pneumonia	POS	4	0
14	M	80	1	Leukaemia	Pneumonia	Congestive cardiac failure	NEG	13	0
15	F	6	1	Nil	Oedematous malnutrition	Pneumonia	NEG	0	1

m- months; d- days; BSI- bloodstream infection; Neg- negative; Pos- positive; UK- unknown; PDA- patent ductus arteriosis; VSD- ventricular septal defect

Table 5.9: Autopsy results by system for 15 children who had bloodstream *Klebsiella pneumoniae* infection

Case	RS	CVS	GIT	CNS	GUS	Skin	Other
1	Intra-alveolar haemorrhages	Congenital PDA, VSD	Congested liver and spleen	Diffuse hypoxic injury	Bilateral cystic dysplasia	Multiple testicular abscesses	Bilateral adrenal haemorrhage
2	Focal haemorrhagic pneumonia	Normal	Congested liver and spleen	Not examined	Normal	Extensive loss of epidermis	DIC
3	Confluent necrosis, multiple pyaemic abscesses	Myocardial haemorrhages	Necrotising oesophagitis, multiple splenic abscesses	Congested	Multiple renal abscesses	Nil	-
4	Multilobar KP BPN, pulmonary haemorrhage	Normal	Oesophageal ulcers, fibrosis, gastritis	Normal	ATN	Nil	DIC, adrenal haemorrhage
5	Haemorrhagic BPN	Normal	Acute primary peritonitis	Not examined	ATN	Multiple septic skin lesions	Minimal activity of DMS
6	Aspiration BPN, septic pulmonary emboli	Endomyocardial haemorrhage	Haemorrhagic pancreatitis	Extensive multifocal cortical haemorrhages	ATN	Nil	DIC
7	BPN, PHPT	Hypertrophied right ventricle	Reactive hepatitis, congested spleen	Not examined	Normal	Nil	Congested adrenals
8	KP BPN, alveolar haemorrhage	Hydropic degeneration	Haemorrhagic infarction/ necrosis	Nil	ATN, mucosal haemorrhage	Skin ulcers	DIC, adrenal haemorrhage

9	BPN with suppurative inflammation	Normal	Invasive candida, diffuse CMV infection	Hypoxic injury	CMV infiltration	Severe excoriation	DIC, disseminated CMV infection
10	Necrotising BPN:KP, measles and PJP evident	Subendocardial necrosis	Oesophageal and gastric ulceration	Not examined	ATN	Normal	Adrenal haemorrhage
11	Extensive necrotising KP BPN	Normal	Necrotising oesophagitis and enterocolitis	Not examined	Numerous thrombi	Punched out skin lesions	DIC
12	Extensive necrotising KP BPN+ empyema	Normal	Hepatic steatosis	Normal	ATN	Napkin dermatitis	DIC, adrenal haemorrhage
13	Extensive KP BPN	Normal	Focal intestinal necrosis	Not examined	Plasmocytic infiltrates	Normal	DIC, adrenal haemorrhage
14	KP BPN	Normal	Not examined	Not examined	Not examined	Normal	Enlarged hilar lymph nodes
15	Diffuse alveolar damage, KP BPN	Flabby myocardium	Hepatic steatosis	Hypoxic injury	Chronic bladder wall inflammation	Excoriated nappy rash	Adrenal haemorrhage

KP- *Klebsiella pneumoniae*; BPN-bronchopneumonia; PDA- patent ductus arteriosus, VSD- ventricular septal defect; DIC- disseminated intravascular coagulopathy; ATN- acute tubular necrosis; PHPT- pulmonary hypertension; PJP- *pneumocystis jirovecii*; CMV- *cytomegalovirus;* DMS- dermatomyositis

5.6 Laboratory results in association with crude mortality

Mortality increased significantly when there was evidence of organ failure or dysfunction concomitant with the BSI. Table 5.10 summarises these data. Mortality was higher in 58/123 (47.2%) children wih renal dysfunction compared to 47/287 (47.2%) children with renal dysfunction who lived, p <0.0001: RR 2.88 (95% CI 2.09-3.97). In the presence of hepatic dysfunction, 49/123 (39.8%) children died compared to 22/287 (7.7%) children who lived, p<0.0001: RR 5.20 (95% CI 3.29-8.20), on univariate analysis.

Table 5.10: The association of organ dysfunction in children with KP bloodstream infection and mortality

Risk factor	All children n=410 (%)	Alive n=287 (%)	Died n=123 (%)	p value; RR (95% CI)
Respiratory failure	144/410 (35.2%)	56/287 (19.5%)	88/123 (71.5%)	<0.0001; 3.67(2.82-4.75)
Renal dysfunction	105/410 (25.6%)	47/287 (16.4%)	58/123 (47.2%)	<0.0001; 2.88 (2.09-3.97)
Hepatic dysfunction	71/410 (17.3%)	22/287 (7.7%)	49/123 (39.8%)	<0.0001; 5.20 (3.29-8.20)
Coagulopathy	73/127 (57.5%)	21/287 (7.3%)	52/123 (42.3%)	0.001; 1.77 (1.23-2.55)

RR: risk ratio

5.6.1 Haematological changes

Children who died had a significantly lower mean haemoglobin of 9.19 g/dl compared to those who survived; survivors' mean haemoglobin was 9.94 g/dl, p value=0.001.

Their median MCV did not differ. The median band, neutrophil, and lymphocyte counts did not differ in the two groups of children; however, the median platelet count of 54 x 10^9/L was significantly lower in the children who died, p=<0.0001.

Infective marker CRP was significantly higher in children who died with a median value of 142(IQR 82-206) mg/l. This is in contrast to the median value of 79 (IQR 27-186) mg/l in those who lived, p= 0.001. Procalcitonin (PCT) levels did not differ significantly (see Table 5.11).

Table 5.11: Comparison of baseline haematological indices in children with KP bloodstream infection who survived or died

Variable	All children (n=410)	n	Alive (n=287)	n	Died (n=123)	n	p value
Mean Hb (SD)	9.71 (± 2.18)	407	9.94(2.08)	285	9.19 (2.32)	122	0.00
Median MCV (IQR)fL	84 (78-89)	407	84 (78-89)	285	84 (78-90)	122	0.82
Median wbc (IQR) x 10^9	11.7 (6.24-20.09)	407	11.7 (6.6-19.9)	285	12.25 (3.69- 22.63)	122	0.92
Median band count (IQR) x10^9	1.50 (0.43-4.16)	354	1.44 (0.45-3.7)	238	1.63 (0.29-4.7)	116	0.58
Percent band count (IQR)	14 (5-27)	243	13 (5-27)	238	16 (6-28)	116	0.58
Neutrophil count (IQR) x10^9	4.66 (1.48-10.32)	355	5.3 (1.86-10.12)	240	3.38 (0.45-11.29)	115	0.25
Lymphocyte count (IQR) x10^9	2.64 (1.27-4.37)	354	2.65 (1.37-4.35)	239	2.62 (1.04-4.55)	115	0.81
Platelet count (IQR) x 10^9	113(34-306)	405	154 (52-356)	283	54(18-160)	122	<0.0001
C reactive protein (IQR) mg/l	108 (38-194)	203	79 (27-186)	137	142 (82-206)	66	0.00
Procalcitonin (IQR) ng/ml	15.25 (1.9-41.7)	106	7.35 (1.6-41.7)	70	22.75 (7.6-41.3)	36	0.16

Hb: haemoglobin
WBC: white blood cell
MCV: mean corpuscular volume
IQR: interquartile range
SD: standard deviation

5.6.2 Analysis of baseline haematological cell line changes and mortality

Red cell line reduction, as represented by anaemia, was the most common single cytopaenia in children who died or survived (see table 5.12). The prevalence of leukopaenia was not significantly different in children who died; however, isolated thrombocytopaenia as well as pancytopaenia were more common in children who died. The bicytopaenias, anaemia with thrombocytopaenia, anaemia with

leukopaenia, and leukopaenia with thrombocytopaenia were all more common in the group that died (see Table 5.12).

Table 5.12: Comparisons of haematological cytopaenias in children who lived or died

All Children	Alive (n=287)	Died (n=123)	RR (95% CI)	p value
Cell line	n (%)	n (%)		
Anaemia (Hb<11)	196 (68.3)	94 (76.4%)	1.11 (0.99-1.27)	0.10
Leukopaenia	62 (21.8%)	34 (27.9%)	1.28 (0.89-1.84)	0.18
Thrombocytopaenia	135 (47.7%)	88 (72.1%)	2.11 (1.50-2.98)	<0.001
Pancytopaenia	34 (12%)	28 (23%)	1.65 (1.20-2.29)	0.00
Thrombocytopaenia, no pancytopaenia	101 (35.2%)	60 (48.8%)	1.38 (1.09-1.76)	0.01
Anaemia with Thrombocytopaenia	98 (34.2%)	73 (59.4%)	2.04 (1.51-2.76)	<0.001
Anaemia with Leukopaenia	49 (17.1%)	32 (26.0%)	1.43 (1.04-1.97)	0.04
Leukopaenia with Thrombocytopaenia	43 (15%)	30 (24.4%)	1.49 (1.08-2.06)	0.02

5.7 Univariate analysis of risk factors of all children who died

Table 5.13 summarises crude risk factors associated with death. KPBSI without a focus was associated with a lower mortality risk in 159/286 (55.6%) than in those patients with an identified focus of infection [47/122 (38.5%); p=0.002; RR 0.61(95% CI 0.45-0.84)]. Factors that on univariate analysis were associated with increased mortality included ESBL KP BSI, HIV infection, being in the PICU at the time of the BSI, KPBSI requiring admission to PICU, and the presence of excoriated skin (see Table 5.13).

Table 5.13: Factors associated with crude inpatient mortality in children with KP bloodstream infection

Risk factors for death	Alive n/N (%)	Died n/N (%)	Univariate analysis RR* (95% CI)	p-value	Adjusted RR* for mortality aRR (95% CI)	p-value
ESBL-KP BSI	228/287 (79.4)	111/123 (90.2)	1.94 (1.13-3.32)	0.01	1.37(0.78-2.38)	0.26
Age (infant vs. >1y)	194/282 (68.8)	88/123 (71.5)	1.14 (0.82-1.59)	0.43	0.88 (0.63-1.23)	0.46
Gender (female:male)	136:151	62:61	0.92 (0.68-1.24)	0.58	-	-
Moderate or severe underweight-for-age	163/287 (56.8)	78/123 (63.4)	1.22 (0.89-1.66)	0.22	1.16(0.83-1.53)	0.45
HIV-infected**	82/288 (28.5)	39/82 (47.6)	1.78 (1.29-2.46)	0.001	1.80(1.32-2.45)	<0.001
Patient in the PICU at time of BSI**	64/287 (22.3)	39/123 (31.7)	1.38 (1.02-1.88)	0.04	1.61 (1.13-2.30)	0.01
Patient needed to go to PICU at the time of KP bloodstream infection**	47/287 (16.4)	37/123 (30.1)	1.67 (1.23-2.26)	0.001	1.70 (1.18-2.45)	0.004
IV fluid infusion >3days prior to BSI	193/284 (68)	84/121(69.4)	1.05 (0.76-1.46)	0.75	-	
BSI without source**	159/286 (55.6)	47/122 (38.5)	0.61 (0.45-0.84)	0.002	0.71 (0.51-0.97)	0.03
Chronic underlying medical condition	176/287 (61.3)	87/123 (70.7)	1.35 (0.97-1.88)	0.07	1.24 (0.87-1.78)	0.24
Excoriated skin**	76/285 (26.7)	54/122 (44.3)	1.69 (1.27-2.26)	<0.001	1.74 (1.31-2.32)	<0.0001

n/N: stratum specific proportions; ** p-value≤0.05; RR: risk ratio.
ESBL KP BSI: Extended spectrum beta lactamase producing *Klebsiella pneumoniae* bloodstream infection
PICU: paediatric intensive care unit
aRR: adjusted risk ratio *: Multivariate model adjusted for age, nutrition, HIV infection, ESBL, Patient in PICU, patient needing to go to PICU, KPBSI without source, chronic underlying medical condition, excoriated skin

5.8 Multivariate analysis of children who died

In the multivariate analysis, HIV infection and excoriated skin remained significant risk factors for death as did patients either in the PICU at the time of the KPBSI or needing PICU admission. Children with KP BSI without a clinical focus were less likely to die. The group of children with excoriated skin included those with burn wounds as a form of extreme excoriated skin. If the children with burn wounds were excluded from the analysis, skin excoriation remained a significant risk factor for mortality [p=<0.0001, RR 1.76 (1.33-2.33)].

5.8.1 Illness-specific factors and mortality

In the crude analysis, children without a clinical focus of infection had a lower risk of death. This is in opposition to those who had advanced organ involvement such as pneumonia, peritonitis, or NEC (see Table 5.14).

Table 5.14: Illness specific factors and unadjusted relative risk of death in 410 hospitalised children with bloodstream *Klebsiella pneumoniae* infection

Illness-specific factors	Alive n/N (%)	Died n/N (%)	Univariate analysis p-value;	RR (95% CI)
BSI without a focus	159/286 (55.6)	47/122 (38.5)	0.002;	0.61 (0.45-0.84)
Pneumonia	74/285 (26)	48/122 (39.3)	0.007	1.52 (1.13-2.04)
Peritonitis	8/285 (2.8)	12/122 (9.8)	0.003;	2.11 (1.43-3.12)
Necrotising enterocolitis	9/286 (3.2)	11/123 (8.9)	0.01;	1.91 (1.25-2.93)

RR: risk ratio
BSI: bloodstream infection

5.9 Complications associated with mortality

Children whose BSI was associated with complications had a higher risk of mortality as compared to those whose BSI was uncomplicated (see Table 5.15).

Table 5.15: Clinical complications and relative risk of death in hospitalised children with bloodstream *Klebsiella pneumoniae* infection

Complication	Alive n/N (%)	Died n/N (%)	Univariate RR RR (95% CI)	p-value
Respiratory failure	56/287 (19.5)	88/123(71.5)	4.64(3.32-6.49)	< 0.001
Renal injury	47/287 (16.4)	58/123 (47.2)	2.59 (1.97-3.42)	< 0.001
Hepatic dysfunction	22/287 (7.7)	49/123(39.8)	3.16 (2.45-4.08)	< 0.001
Septic shock	48/284 (16.9)	91/122(74.6)	5.64 (3.96-8.02)	< 0.001
Coagulopathy	21/53 (39.6)	52/74(70.3)	1.75 (1.23-2.49)	0.001
Patient needed to go to PICU	47/287 (16.4)	37/123 (30.1)	1.67 (1.23-2.26)	0.002

RR: relative risk
PICU: paediatric intensive care unit

Crude mortality included all children with KPBSI who died including 28/123 (22.8%) who died more than 14 days after the BSI, implying that these deaths were not associated with the BSI. Analysis of deaths occurring within the first 14 days of BSI had a higher chance of identifying risk factors for KPBSI.

5.9.1 Factors associated with mortality within the first 14 days of KPBSI

An exploratory analysis of factors associated with mortality was unable to distinguish any difference in children who died within the first 14 days of the KPBSI from those who died later (see Table 5.16).

Table 5.16: Factors associated with mortality <14days of KP bloodstream infection

Risk factors for mortality <14 days (n=95 (77.2%))	Univariate analysis p-value; RR (95% CI)
ESBL-KP bloodstream infection	0.10; 1.36 (0.83-2.21)
Age (infant vs. >1y)	0.33; 1.11 (0.88-1.41)
Gender (female:male)	0.36; 0.91 (0.75-1.11)
Moderate or severe underweight	0.74; 1.04 (0.84-1.27)
Hospitalisation within previous 28 days	0.6; 1.05 90.87-1.27)
HIV-infection	0.67; 0.95 (0.75-1.20)
Concomitant chronic medical condition	0.13; 0.85 (0.71-1.02)
Patient in the PICU at time of B/C	0.33; 0.90 (0.72-1.13)
Patient needed to go to PICU at the time of KP bloodstream infection	0.50; 1.07 (0.88-1.31)
Bloodstream infection without source	0.33; 0.90(0.73-1.12)
Time to effective antibiotic	0.13; 0.85 (0.71-1.02)
Excoriated skin	0.55; 1.06 (0.88-1.29)

ESBL-KP: Extended-spectrum β-lactamase producing-*Klebsiella pneumoniae*
RR: risk ratio
PICU: paediatric intensive care unit
B/C: blood culture

5.9.2 Factors associated with mortality within the first 3 days of KPBSI (early KPBSI deaths)

With the 57 (46.3%) children who died within 3 days of the KP BSI, the mean time to death in this group was 1 (SD ± 0.93) day. Twenty-one children died on the day healthcare providers took the blood culture. Two children died on the day of hospital admission: one of them had a community-acquired ESBL-KP infection and the other

had healthcare-associated ESBL-infection; the HIV status was unknown in both children.

Of the children dying within three days of KP BSI, 23 (41%) presented with diarrhoeal disease, 15 (27%) had pneumonia, 13 (23%) had kwashiorkor, and 9 (16%) were thought to have *sepsis*. Univariate analysis did not identify specific risk factors identified in this group of children that died within 3 days of the positive blood culture. Those who were already in the PICU setting prior to the BSI were less likely to die within 3 days of the infection; thus, researchers could not generate a multivariate analysis (see Table 5.17).

Table 5.17: Factors associated with early (patient died within 3 days of KP bloodstream infection) mortality

Risk factors for early (death≤ 3days) mortality (n=57 (46.3%))	Univariate analysis p-value; RR* (95% CI)
ESBL-KP bloodstream infection	0.34; 1.43 (0.63-3.26)
Age (infant vs. >1y)	0.48; 0.86 (0.58-1.28)
Gender (female: male)	0.92; 0.98(0.67-1.44)
Moderate or severe UWFA	0.75; 1.07 (0.71-1.59)
HIV-infection	0.59; 1.11 (0.75-1.64)
Patient in the PICU at time of B/C*	0.02; 0.57 (0.34-0.96)
Patient needed to go to PICU at the time of KP bloodstream infection	0.13; 1.35 (0.93-1.97)
Bloodstream infection without source	0.83; 0.96 (0.64-1.43)
Time to effective antibiotic	0.27; 0.79 (0.53-1.18)
Excoriated skin	0.66; 1.09 (0.74-1.60)

ESBL-KP: Extended-spectrum β-lactamase producing *Klebsiella pneumoniae*
RR: risk ratio
PICU: paediatric intensive care unit
B/C: blood culture
UWFA: underweight-for-age
* p-value ≤0.05

CHAPTER SIX: DISCUSSION

This hospital-based retrospective study helped describe the emerging problem of multi-drug resistant *Klebsiella pneumoniae* (KP) bloodstream infections (BSI) at Red Cross War Memorial Children's Hospital (RCWMCH). The study aim was broken down into six specific objectives. (1) The description of the presentation of *Klebsiella pneumoniae* (KP) bloodstream infections (BSI) at RCWMCH over a 6 year period. (2) The description of the spectrum of risk factors associated with ESBL- *KP* BSI. (3) The comparison of the healthcare-related- and community-acquired *KP* BSI. (4) The evaluation of the antibiotic susceptibility patterns of Klebsiella pneumoniae isolates and the antibiotic selection and response to KP-bloodstream infections. (5) The evaluation of factors associated with inpatient mortality. Finally, (6) the assessment of the outcome of *KP* BSI in HIV-infected and uninfected children. This chapter contains a discussion of each of these objectives. Thereafter, there is an analysis of limitations and strengths of the study.

6.1 Objective one: Presentation of KP BSI at Red Cross War Memorial Children's Hospital over a 6 year period 2006-2011

6.1.1 Patient profile

The study contained a skewed age distribution documenting that infants were the most affected age group. The median age of the children was 5.0 months, interquartile range (IQR) 2 to 16 months, with 68.9% (282) being less than 12months old. In the only other large published paediatric case series on *KP BSI* that involved 57 American children, infants were also the most vulnerable age group, in that study-68% (n=38/57) of the children were less than 12 months (Bonadio, 1989). In the Korean study by Kim et al., the mean (±SD) age of the study children with ESBL-producing

Escherichia coli (ESBL-EC) and ESBL-producing *KP* BSI (ESBL-KP) was 4.58 (±5.02) years (Kim et al., 2002). Zaoutis et al., reported a median age (IQR 0-11) of 2 years in their study on children with ESBL-EC and ESBL-KP BSI (Zaoutis et al., 2005). In a Kenyan study on paediatric community-acquired BSI, 37% (6137/16 570) of children were less than 12 months (Berkley et al., 2005). In that study, *Klebsiella spp.* made up only 3.2% (36/1132) of the community-acquired bloodstream isolates. Possibly the major reason for this observation is that a high proportion of all children admitted to RCWMCH are under 12 months of age, in two 12-month-long surveys conducted in 2007 and 2010 in one of the general medical wards showed that 66.7% (IQR: 59 – 76) and 62.1% (IQR: 53.9 – 69.2) respectively of all admitted children were < 12 months of age (Weakley et al., 2009 and B Eley, personal communication, 29th July 2015) Another important contributory reason may be that young children, particularly infants, are not fully immunocompetent and may be susceptible to infectious diseases including KP BSI; this may also contribute to the delayed diagnosis of sepsis (Clapp, 2006).

The prevalence of child malnutrition in the less than 5-years age group in sub-Saharan Africa, defined as < -2 WFA z-score, was 8.7% in 2012. In South Africa, the estimate was at 9%. (UNICEF, New York; WHO, Geneva; The World Bank, Washington, DC; 2012). A National Foods Consumption survey of 2005 that found that 1 in 5 South African children were stunted and 1 in 10 were UWFA (National Food Consumption Survey Fortification Baseline (NFCS-FB-I) South Africa, 2005). The present study however documented a high prevalence of underweight in hospitalised children. In this study, 241 (58.8%) children were moderately to severely underweight-for-age. Of these, 163 (39.8%) children were severely underweight-for-age (UWFA). Children with with moderate- or severe- underweight-for-age were at higher risk of having

ESBL-KP (209/339, 61.7%) infection compared to those with non-ESBL infection (32/71, 45.1%); p=0.01. Malnutrition is associated with widespread micronutrient deficiencies, immune dysfunction, and susceptibility to infectious complications (Beisel, 1996).

Documentation showed HIV status in 70% (288/410) of study children. Of the children, 206 (50.2%) were HIV negative and 82 (20%) were HIV-infected. This is important information given the burden of HIV disease in Sub-Saharan Africa. It allowed unique insight into not only the effect of HIV infection on KP BSI but also the effect that frequent contact with the healthcare environment had on the development of healthcare-related KP BSI. UNAIDS estimate that there are some 3 million children aged 0-14 years living with HIV in sub-Saharan Africa; the prevalence in South Africa is estimated at 410 000 children (UNICEF, 2013). The high prevalence of HIV infection shown in the study children likely reflected the degree of immunosuppression that characterises paediatric HIV disease and the susceptibility to BSI and need for hospitalisation. Consistent with this, Le Roux et al. reported on 47 BSIs occurring in a cohort of 125 HIV-infected children (Le Roux et al., 2011). Furthermore, in the present study, HIV infection was associated significantly with ESBL infection compared with non-ESBL KPBSI on univariate analysis (p =0.001, RR (RR 1.17, (95% CI 1.08-1.26)). Only 3/82 (3.7%) HIV-infected children had non-ESBL infections.

6.1.2 Clinical site of infection at the time of the KP BSI

There were differences noted at presentation in children with and without ESBL-related KP BSIs at the time of the BSI. ESBL-KP infections were more common in children who had pneumonia as their primary manifestation (p=0.03; RR 1.65 (95%CI 1.00-2.70)). Children with non-ESBL-KP infection were less likely to have an identifiable clinical focus (p= 0.004; RR 0.72 (95% CI 0.59-0.88)). Other clinical events

were seen at the time of the blood culture including peritonitis, necrotising enterocolitis (NEC), soft tissue and wound infections, urinary tract infections (UTI), septic peripheral drip sites, and central vascular catheters. These did not significantly link to ESBL-infection. In contrast, Kim et al. found that 42% of their study children with non-ESBL Gram-negative BSI had clinical foci of infection compared to 27% of those with ESBL-BSI; however, these rates did not reach statistical significance, p=0.07 (Kim et al., 2002). Although 50.2% (206/410) of the children in the present study did not have a definable clinical focus at the time of the BSI, when found this was typically in the lungs- 29.7% (121/410) of the children with a clinical diagnosis of pneumonia. In a parallel study on Staphylococcus bacteraemia at the same institution, 33% (110/337) of children had no identifiable source and, where identified, the lungs were the most common focus in 72/337 (22%) children (Naidoo et al., 2013). Other paediatric studies of Gram-negative BSIs including *KP* described bacteraemia in the absence of a clear clinical focus; the prevalence was 68% in one study (Levy et al., 1996).

The infection was nosocomial or healthcare-associated in 389/410 (95%) children and community-acquired in 21 (5%). The striking scarcity of the resistant ESBL-form from the community and the predominance of nosocomial acquisition of the ESBL-KP is consistent with other reports (Levy et al., 1996; and Al-Hasan et al., 2011).

The inflammatory process involved in an episode of BSI is complex. Unlike the child coming in from home with the CA BSI where the BSI is already manifest by virtue of presentation at a healthcare facility, there may be delays in recognition of the BSI in the child with a HRF-BSI. In addition children may have non specific signs of sepsis leading to delays in recognition especially if they have been hospitalised with another clinical complaint. In the present study 206 (50.2%) of all children did not have a

definable clinical focus of infection and, 30% (126/410) did not experience fever at the time of the positive blood culture; ineffective empiric therapy would also lead to further delays. In a meta-analysis comparing the mortality of adults and children with ESBL-related and non-ESBL-related *Enterobacteriaceae* BSI, Schwaber and Carmeli described significant association between ESBL production by *Enterobacteriaceae* and delays in effective therapy (Schwaber and Carmeli, 2006). The present study found the median hospitalisation time from admission to non-ESBL BSI (3days) was significantly shorter than that for the acquisition of ESBL-BSI (9days), p<0.0001, RR 1.45 (1.21-1.74) but was limited by the fact that time to antibiotic therapy was not known because of incomplete documentation in the clinical case files.

6.1.3 Changes in laboratory parameters at the time of the KP BSI

In keeping with the high prevalence of malnutrition in the study children, 71% (290/407) of patients had normocytic normochromic anaemia at the time of the bacteraemic event. The mean haemoglobin was 9.7 g/dl SD± 2.18 and the median mean corpuscular volume (MCV) was 84 (IQR 78-89) femtolitres. However, anaemia was not more prevalent in children who were moderately or severely UWFA compared with those with normal weight-for-age, p=0.75; RR 0.97 (95%CI 0.81-1.16). Furthermore the study also showed that anaemia was not more prevalent in 183/263 (69.6%) children with a chronic underlying condition compared to 107/147 (72.8%) children without, p= 0.49. In contrast, a National Foods Consumption Survey of 2005 found that one third of South African children aged 6-71 months were anaemic (National Food Consumption Survey Fortification Baseline (NFCS-FB-I) South Africa, 2005).

Children with ESBL-KP infection had a lower mean haemoglobin, 9.57 g/dl versus 10.5g/dl, p=0.003, and lower median platelet counts, 98 (IQR 31-284) versus 183

(IQR73-382), p=0.003, compared with children with non-ESBL KP BSI. Their median white blood cell (WBC) counts and percentage band counts did not differ significantly. Thrombocytopaenia is commonly seen in bacteraemic processes with this commonly due to a consumptive process (Goyette et al., 2004). It is not clear why an ESBL-KP BSI associated with a lower platelet count compared with having a non-ESBL infection. Particularly since the median values of the two pro-inflammatory markers C-reactive protein (CRP) and procalcitonin (PCT) were elevated in both groups, 108 (IQR 38-194) and 15.25 (IQR 1.9-41.7) respectively. Elevated levels of CRP >80 mg/l and PCT >2ng/ml are commonly regarded as better cut-off values for identifying invasive bacterial infections as opposed to lower levels that may sometimes be seen with viral illnesses (Van den Bruel et al., 2011). In the children tested, 59.1% (120/203) and 74.5% (79/106) had levels of CRP and PCT above these values; this was uninfluenced by the ESBL status of the KP isolate.

Coagulation disturbances were evident in 17.6% (73/127) of the tested children; 61.1% (66/108) of those with ESBL-KP infection had evidence of disturbed coagulation compared with 36.8% (7/19) with non-ESBL infection, p=0.05: RR 1.16(1.00-1.37). Coagulation disturbances may reflect the degree of clinical complications that can be associated with invasive Gram-negative infections. It is unclear why having a resistant organism predisposes to inflammatory mediated coagulation disturbances but may rather reflect treatment delays. For example the study documented that 94.3% (66/70) of children with non-ESBL BSI received an appropriate empiric antibiotic compared with 74.5% (241/328) who had ESBL-KP BSI, p=0.0002, this may imply treatment delays. However, this is only speculation because the study did not document the time delay between drawing the definitive blood culture and the introduction of the empiric antibiotic.

6.2 Objective two: Spectrum of risk factors for ESBL-acquisition

Using generalised linear modelling with Poisson distributions, the adjusted analysis showed significant risk factors for the acquisition of ESBL-KP BSI. Risk factors included cephalosporin exposure in the preceding 12 months prior to the KPBSI, HIV infection and more than 3 days IV fluid infusions prior to the KP BSI.

Prior cephalosporin exposure appears to be a common risk factor described in several paediatric studies involving the broader group of *Enterobacteriaceae* BSIs. As in the present study, Zaoutis et al. in the USA and Kim et al. in Korea showed that cephalosporin exposure significantly increased the risk of ESBL acquisition, p= 0.002; OR, 5.82 (95% CI ,1.92–17.68) and p = 0.001; OR, 5.56(95% CI, 1.9 to 16.0) respectively. Tsai et al. in Taiwan reported this association with cephalosporin exposure as significant in neonates, and Ariffin et al. in Malaysia reported this as well as in children with febrile neutropaenia and underlying oncological malignancies (Ariffin et al., 1999; Tsai et al., 2014).

Underlying renal disease and duration of total parenteral nutrition were further significant neonatal risk factors for the development of resistance in Gram-negative BSI (Samanta et al., 2011; Tsai et al., 2014). Kim et al. also reported prior hospitalisation and admission to PICU within the previous month in their study (Kim et al., 2002). In the present study, these two factors did not seem to be significant. Certain unique factors included in the adjusted multivariate model may have influenced this.

There were several factors described in the present study that were notably different from those described in adults and children in developed countries. These factors included HIV-infection and duration of intravenous (IV) fluid infusions for longer than

3 days prior to the BSI that were associated with the development of ESBL-*Klebsiella pneumoniae* (ESBL-KP) BSI infections. No other paediatric studies have specifically described KP BSI in HIV-infected children.

The issue of prolonged intravenous fluid infusions as a risk factor for *ESBL-KP* infection could include many possible reasons. For instance, the risk could relate to contamination of the cannula at or following intravenous insertion, contamination of the administration sets, and a break in protocol of ensuring sterility when changing administration sets. The risk could be due to contamination of intravenous fluids when healthcare providers use lines for other functions such as administering intravenous drugs or the practice of not changing the administration sets after specified periods, particularly in those who have spent long periods in hospital.

Koerner et al., who implicated improper infusion preparation and irregular staff changes, previously described outbreaks of septicaemia from gram-negative-contaminated intravenous infusions in cardiology patients (Koerner et al., 1997). Another case series from Mahatma Gandhi Memorial Hospital, a public neonatal unit in KwaZulu-Natal, involved 26 neonates with CVPs and intravenous fluid infusions. An infection control examination identified KP-contaminated TPN solutions, solutions that were sterile on opening but accessed multiple times against procedural guidelines by repeated needle insertion to minimise waste. The mortality rate was extremely high at 85% (Moodley et al., 2005). None of these other studies have shown what the present study has shown in a multivariate model i.e., intravenous cannulation to be a significant risk factor for ESBL-acquisition. The significance may simply reflect poor infection control measures.

An environmental inspection at RCWMCH's short stay unit during a one of the diarrhoeal seasons did not find that any surface-colonisation with *KP*. Although there

was no specific testing of any flowing intravenous infusions, it is plausible that this mechanism of infection may be more common than commonly thought in wards that are overcrowded and under-resourced. In these situations, work-related pressures facing the ward nurses do not allow for full compliance with infection control measures. One should also acknowledge that the point of entry through the skin of an intravenous cannula remains an area of broken skin, and gut pathogens could thus contaminate or colonise this localised loss of skin integrity. This mechanism may be important in any hospitalised children requiring intravenous cannulae for any length of time.

HIV infection was also a significant risk factor for the infection with resistant KP, p-value 0.01: RR 1.12 (95% CI 1.02-1.22); there were 79/82 (96.3%) HIV-infected children with resistant KP compared to 3/82 (3.7%) with non-ESBL KP. There may be several reasons for this. HIV-infected children are in frequent contact with the healthcare system, require more hospitalisations than HIV-uninfected children require, and are prone to infectious complications by virtue of their immunosuppression. For these reasons, they may easily become colonised with resistant organisms and still be vulnerable despite antiretroviral therapy (ART). Colonisation may have important implications for the development of nosocomial BSIs in children. In one study, nasopharyngeal aspirate (NPA) specimens from 167 HIV-infected South African children attending a central hospital outpatient clinic showed that about 15% (n=32) of children were colonised with *Enterobacteriaceae* with 50% being ESBL producers (Cotton et al., 2008). It was not known which patients in the present study were colonised with KP or any other pathogens as rectal swabs or stool cultures were not routine on admission.

6.3 Objective three: Comparison of healthcare related and community-acquired KPBSI

In this study, the adjusted analysis showed that children with healthcare-related KP BSI were more likely to have ESBL-KP BSI, a concomitant chronic medical condition or be in the PICU at the time of the KP BSI. It is evident that BSI with KP is most likely a nosocomial event. Most of the children, 95%, had healthcare risk factors; the infection was nosocomial or healthcare-associated in 389 children and community-acquired in only 21 (5%). Other studies also strongly suggest that this problem of *KP* BSI is a product of the healthcare environment. Reddy et al. showed that *Klebsiella spp*. caused 2.8% of the BSI caused by *Enterobacteriaceae* in their meta-analysis of community-acquired infections across 22 studies (Reddy et al., 2010). Berkley et al., from Kenya, reported that KP ranked ninth out of ten in the causative aetiology of community-acquired BSI in 1094 Kenyan children (Berkley et al., 2005). In another Kenyan paediatric study, Aiken et al. reported that KP caused 20% (43/353) of nosocomial BSI but only 2% (37/1590) of the community-acquired infections.

The high prevalence of a concomitant chronic medical condition in 262 (64%) children indicated high exposure to the health system and that the problem of KPBSI is a healthcare related one. Consistent with the present study, an American paediatric study reported that 91% of children with nosocomial or healthcare-associated Gram-negative BSI were more likely to have an underlying medical condition compared to 32% with community-acquired BSI , $p<0.001$(Al-Hasan et al., 2011). Furthermore, they may also indicate that the carriage of these resistant strains is prolonged. Researchers have shown this in several studies including that by Löhr et al. where the median duration of faecal carriage in 51 infants discharged from a neonatal intensive care unit was 12.5 (IQR 9-17.5) months, with the longest time of 23.5 months (Löhr

et al., 2013). Whilst septic shock may place children into the PICU environment, time spent among critically ill children in the PICU may more commonly predispose them to invasive nosocomial infections. This may happen because of supportive procedures such as mechanical ventilation, central venous catheterisation, urinary catheterisation, invasive monitoring, and invasive surgery.

Children admitted with community-acquired KP BSI were significantly younger. For these, the median age was 1.5 (IQR 0.7-3.8) months compared with 5.5 (IQR 1.9-16.2) months in those with healthcare-related KP BSI. This may relate to the susceptibility to serious bacterial infections including KP in very young infants with immature immunity brought into the healthcare environment (WHO, 1999). There were 7 (1.7%) cases of community-acquired ESBL-KP BSI - confirmimg that the resistant organism was present in the community during the study period.

6.3.1 Haematological differences in children with nosocomial versus community-acquired KPBSI

At the time of the KP BSI, children with healthcare-associated risk factors (HRF) had a significantly lower mean haemoglobin value at 9.65 ±2.18 g/dl than 10.6 ±1.89 g/dl seen in children with community-acquired KP BSI, p-value=0.02. Whilst the median WBC and percentage band counts did not differ significantly, children with HRF had a lower median platelet count of 103 (IQR 32-288) $x10^9$/l compared with 350 (IQR 193-659) $x10^9$/l seen in those children those whose infection was community-acquired, p-value= 0.0001. The anaemia witnessed in children with HRF may reflect a number of processes such as marrow suppression that is commonly seen in sick children or may even be iatrogenically-induced from repeated venesection. Thrombocytopaenia likely reflects a consumptive process that frequently accompanies serious Gram-negative BSIs and may be more evident in hospitalised children because of delays in

recognising the onset of the infection as other processes may be occurring concomitantly.

6.3.2 Differences in mortality in children with healthcare-related KP BSI compared with community-acquired KP BSI

There was no significant difference in the mortality risk or median time to death from the time of the positive KP blood culture in children with HRF compared to those with community-acquired BSI. In contrast, Levy et al. reported that hospital-acquired Gram-negative bacteraemia accounted for 48.5% of 374 episodes in an Israeli paediatric study and that mortality was higher in those with nosocomial BSI compared with community-acquired BSI, 23% versus 11%, p-value=0.006 (Levy et al., 1996). In a Kenyan study Aiken et al. reported that the case fatality rate in the community-acquired group was less than half of that in those with nosocomial BSI, 24% versus 53% (Aiken et al., 2011). BSI with KP, regardless of whether healthcare-related or community-acquired, are serious events and as this study has shown the clinical outcome can be identical.

6.4 Objective four: Antibiotic susceptibility patterns of Klebsiella pneumoniae isolates and the antibiotic selection and response to KP-bloodstream infections

6.4.1 Treatment considerations for children with community acquired (CA) KP BSI

Bloodstream infections with KP, regardless of whether ESBL-associated or not, are serious events. As this study showed, many children died and or experienced adverse complications. Therefore, empiric antibiotic choices have important implications for patient survival, and healthcare providers should make choices using the best available evidence-based knowledge. The South African Department of Health

hospital level paediatric guidelines recommend ceftriaxone for infants and children to cover community acquired sepsis (DOH SA, 2013) whereas the RCWMCH Antibiotic guidelines recommend a combination of ampicillin and gentamicin (Nuttall et al., 2012). The intention for these recommendations is to provide cover for both common Gram-positive organisms and common Gram-negative organisms. This study allowed some insight into what empiric antibiotics were appropriate for KP BSI at RCWMCH, albeit a single pathogen.

6.4.2 Treatment considerations for children with healthcare-related KP BSI

Whenever a patient develops systemic symptoms due to a suspected hospital acquired bacterial organism, it is traditional practice to start with broad cover after appropriate investigations to ensure appropriate antibiotic cover for the likely pathogen. When cultures results become available, the healthcare provider would de-escalate to an appropriate single agent. One should remember that ESBL- producing *KP* and *Escherichia coli* are just two of several resistant hospital-acquired organisms threatening hospitalised children. Other significant organisms at RCWMCH include methicillin resistant *Staphylococcus Aureus (MRSA)*, multi-drug resistant (MDR) *Pseudomonas Aeruginosa,* and resistant *Acinetobacter spp*. Individual patient risk factors should be carefully considered and an appropriate strategy by a focused body involved in the individual patient's care should be laid out. Prolonged hospitalisation especially in intensive care and burn units complicate antibiotic choices further. Healthcare-related BSI episodes can be recurrent and pose daunting challenges.

6.4.2 KP isolate susceptibility patterns

It was not possible to report on time to empiric or definitive antibiotic administration as healthcare providers documented the infection poorly in the case files. On the other

hand, it was possible to equate antibiotics introduced at the time of the definitive blood culture as the empiric antibiotic. The empiric antibiotic prescribed in response to the BSI was appropriate in 77.1% (307/398) of children, 241/398 (73.5%) children with ESBL KP BSI and 66/70 (94.3%) children with non-ESBL KP BSI. Once the laboratory results became available, the healthcare providers adjusted the antibiotic cover, and this delay did not increase crude mortality p=0.29, RR 0.83 (95% CI: 0.59-1.16), 87/307 (28.3%) children on appropriate antibiotics died versus 31/91 (34.1%) children on inappropriate empiric antibiotics.

6.4.2.1 KP isolate susceptibility patterns for CA KP BSI

This analysis showed that at RCWMCH, this combination, ampicillin plus gentamicin, would not have provided adequate cover for 28.7% (n/N= 15/21) children with possible community-acquired KP BSI. However, a combination of ampicillin plus amikacin would have improved cover to 90.5% (n/N= 19/21) of children. The antibiotic susceptibility patterns of the KP isolates were analysed in two periods 2006-2008 and 2009-2011. It was hoped that this would provide some insight into the evolution of resistance over the 6 year study period, however the numbers included in the present study were small. In the period 2006-2008 antibiotic choices of ampicillin with gentamicin, or cefotaxime alone would have provided inferior cover for 54.6% (n/N=6/11) and 45.5% (n/N=5/11) KP isolates compared to ampicillin plus amikacin, ceftriaxone plus amikacin or ertapenem alone where isolate cover was 90.9% , 90.9% and 100% respectively. In the period 2009-2011, numbers were again small, ampicillin plus gentamicin, ampicillin plus amikacin, ceftriaxone plus amikacin, would all have been effective for 90% of isolates and ertapenem would be effective for 100% of isolates. The numbers involved were too small to allow any firm conclusions.

6.4.2.2 KP isolate susceptibility patterns for healthcare-related KP BSI

Analysis of the KP isolates susceptibility patterns over the years 2006-2008 and 2009-2011 showed that antibiotic regimens containing gentamicin or cefotaxime intended for Gram negative cover for healthcare related infections were inferior in that <20% of isolates were susceptible, however this was not relevant for RCWMCH. Over the two time periods cover provided by amikacin deteriorated from 87.9% (n/N=152/173) to 64% (n/N=121/189). Piptazobactam with amikacin combination cover for KP isolates dropped from 91.9% (n/N=157/171) to 81.6% (n/N=142/174). It was also evident that cover provided by ertapenem was not guaranteed- from 100% (n/N=169/169) susceptible in the period 2006-2008 to 98.9% (187/189) in the second half of the study period. From these results, in the case of critically ill children at RCWMCH, one cannot recommend empiric antibiotic combinations of ceftriaxone and amikacin or piptazobactam and amikacin, which were used at RCWMCH for empiric cover for presumed HAI during the study period.

In one study, antibiotic susceptibility of *KP* across public sector sentinel sites in South Africa ranged from 85-98% susceptibility to amikacin, 38-79% for piptazobactam, 20-29% for gentamicin, 91-100% for ertapenem. Clinical data were not reported in these annual reports; however, early signs of carbapenem resistance were becoming evident (Perovic et al., 2013). Ertapenem does not provide adequate anti-pseudomonas activity making empiric antibiotic selection for HAIs a complex issue. The likely range of offending organisms should be carefully considered and linked to multiple factors.

The need for prudence is not only driven by fiscal constraints but also by the threat of carbapenamase resistance, which has now complicated matters further (Nordmann

et al., 2009; Perovic et al., 2013). These include the danger imposed by other multi-drug resistant hospital-acquired infections. Ariffin et al. reported that carbapenem antibiotics were the most effective against ceftazidime-resistant KP BSI in children in an oncology unit with febrile neutropaenia if they were given within 48 hours of developing the BSI. At the same institution researchers showed that over 8 years the resistance trends of KP isolates to ceftazidime and amikacin were very similar and ranged between 21 and 55 % resistance, thus limiting their usefulness in critically ill children (Ariffin et al., 1999). Kim et al. reported that outcomes were less favourable in children with ESBL-producing Gram-negative BSI treated with aminoglycosides compared with children who had non-ESBL infections, the bloodstream isolates were taken from Korean children hospitalised from 1993-1998. Imipenem was used for 10 children- 7 had ESBL-producing *Escherichia coli* or KP BSI and 3 had non-ESBL Gram-negative BSI, 2 of the children with ESBL infection did not survive (Kim et al., 2002). Currently many units would now favour the effectiveness of a carbapenem antibiotic as first-line treatment for a serious KP BSI.

6.5 Objective Five: An evaluation of factors associated with inpatient mortality

6.5.1 Case fatality rate

KPBSI constitutes a serious medical condition with high mortality; this study revealed a case fatality rate of 30% (123/410) children died with 77% (95/123) of deaths occurring within 14 days of the definitive blood culture. In published paediatric studies of Gram-negative BSI including *KP*, researchers also documented high mortality, ranging from 13 to 36% (Kim et al., 2002; Zaoutis et al., 2005; Velaphi et al., 2009; Samanta et al., 2009; Bonadio, 1989). The fact that 46% (57/123) of the deaths

occurred within 3 days of the blood culture speaks to the rapidity of onset of organ failure in patients with *KP* septicaemia. The deaths occurred anywhere in the interval from day the healthcare providers drew KP blood culture to 73 days after. It was assumed that deaths occurring within 3 days of the positive blood culture related to the BSI. Berkley et al. reported similarly that 70.5% (217/308) deaths in rural Kenyan children with bacteraemia occurred within 3 days of positive blood culture (Berkley et al., 2005). Consistent with this, 63% (19/30) of neonates' deaths occurred within 48 hours of KP BSI (Velaphi et al., 2009). Naidoo et al. reported that 75% of 32 deaths occurred within 7 days in children with *Staphylococcus aureus* BSI at RCWMCH. Marra et al. reported on 15-day mortality in nosocomial KP BSI in a study involving adults and children in Virginia, USA; in that study 26/108 (24.1%) of the patients died within 15 days of the blood culture.

6.5.2 Factors associated with mortality in children with KP BSI

In the multivariate analysis using generalised linear modelling, the study revealed excoriated skin, HIV infection, being in the PICU, or needing PICU admission as significant risk factors for death. It also showed that children with KP BSI without clinical focus were less likely to die, p value= 0.03: RR 0.71 (95% CI 0.51-0.97). This is in comparison with children who had a clinical focus of infection.

The limited number of published paediatric studies reporting on KP and other Gram-negative BSI lack multivariate mortality risk factor analyses. Kim et al. identified ESBL production 26.7% versus 5.7 %, p=0.001, and the presence of shock on admission in 40% versus 9.4% in those without shock as significant risk factors for death, p-value=0.001 (Kim et al., 2002). Velaphi et al., who reported a case fatality rate of 30%, found higher mortality of 42% versus 25% in neonates receiving inappropriate antibiotics compared with appropriate cover as a risk factor (Velaphi et al., 2009).

Ariffin et al., who reported higher mortality in those with appropriate treatment delays beyond 48 hours, supported a similar result (Ariffin et al., 1999). In contrast, Tsai et al. reported other infectious complications after the onset of BSI and pulmonary hypertension as independent risk factors for mortality in neonates with Gram-negative, including KP, BSI in Taiwan (Tsai et al., 2014).

6.5.2.1 Excoriated skin

The present study finding of excoriated skin as a significant risk factor for mortality in a paediatric patient population was novel and has significant clinical importance. In the present study, 27 children were admitted with burn wounds. However, there were also 130 (32%) children with areas of excoriated skin sufficient to merit documentation by the caring nurses and doctors. In this study, excoriated skin remained an independent potential risk factor for mortality in the adjusted analysis. Other researchers have shown that invasive burn-wound infections are a serious complication of paediatric burn patients and contribute significantly to their demise (Sheridan, 2005). Multidrug resistant nosocomial Gram-negative bacteria including KP have been implicated in the aetiology in resource poor countries (Bhat and Vasaiker, 2010; Komolafe et al., 2003).

There are few published paediatric studies, but one could reasonably speculate that the breakdown of the integrity of normal skin barriers under certain circumstances could lead to invasive bacterial infections, particularly with enteric colonisers. A retrospective paediatric study from RCWMCH, Cape Town involving 112 children with meningococcal-associated purpura fulminans found *KP* and other organisms contaminating necrotic wounds (Numanoglu at al., 2007). This finding is relevant to developing countries because poor nutrition as well as micronutrient deficiencies such as zinc deficiency adversely affected skin integrity (Kumar et al., 2012). The

study was not conclusively able to demonstrate increased mortality linked to moderate or severe underweight; however, infection is recognized as one of the risk factors for mortality in children with acute severe malnutrition (WHO, 2012). Some research with newborns has been carried out where maintaining skin barrier integrity to reduce infection has shown some success in reducing mortality by 26% and 32% in 497 preterm infants. In this study, researchers randomised the infants to receive one of two skin emollients versus the control group who did not receive emollient care (LeFevre et al., 2010). Broken skin areas may represent significant illness in patients exhibiting skin failure as part of a coexisting multiorgan dysfunction syndrome (MODS). Poor skin integrity is not amenable to laboratory testing but assessed by gross inspection for extent and depth of skin necrosis (Irvine, 1991; Langemo and Brown, 2006).

6.5.2.2 Children in need of PICU support

Children in need of PICU support are typically critically ill, and it is not surprising that mortality in this group of children increases. In this study, children with KP BSI experienced a number of organ dysfunctions because of the septicaemic process. These dysfunctions included respiratory failure, acute renal and hepatic dysfunction, and coagulation disturbances. Mortality increased in children with any of these clinical complications combined with KP BSI, p=0.0001. One can predict outcome measures including mortality in PICU patients using a validated score known as the paediatric logistic organ dysfunction (PELOD) score, which utilises a number of physiological and laboratory measures of organ function. As such, any organ dysfunction contributes to the PELOD score and can help predict mortality in PICUs (Leteurtre et al., 2003).

6.5.2.3 The effect of HIV infection

A separate, dedicated section under the subtitle of Objective 6 includes a thorough discussion of the significant effect from HIV infection on the outcome of KP BSI.

6.6 Objective six: Effect of HIV infection on the outcome of children with KP BSI

Routine testing of all children for HIV infection admitted into the non-surgical wards has always been strongly encouraged at RCWMCH. Despite this, in the present study, the HIV-status was known in 288 of 410 children (70.2%), the reports showed 20% (82) to be HIV-infected. In the present study's 82 HIV-infected children, diarrhoeal disease in 52/82(63.4%) children, and pneumonia in 35/82 (42.7%) were the two most common presenting diagnoses. These infectious diagnoses are common presenting complaints in HIV-infected children from Sub-Saharan Africa. Crude mortality was higher in HIV-infected children compared with uninfected children (RR 1.91 (1.40-2.62), $p<0.0001$)). Of the 82 children known to be HIV-infected, 39 (47.5%) children died; 41 (50 %) children were on ART at the time of the KP BSI. The median time on ART before the KP BSI was 19 (IQR 8-66) days; even though ART may have provided some protection against death ($p=0.02$; RR 0.56 (CI 0.34-0.92)), the mortality of HIV-infected children with KP BSI was high throughout the study period. There was 1/ 5 (20%) child who died who had been on ART for 6 months or longer compared to 13/36 (36.1%) children who died and had been on ART for less than 6 months, $p= 0.44$. This may reflect the severity of this particular Gram-negative BSI in the presence of HIV infection. Early ART has been shown to improve survival and immunity in infants (Cotton et al., 2013). There are limited numbers of African paediatric studies providing information on the effect of HIV infection on the epidemiology KP BSI in hospitalised children, the numbers in the present study were

small. The study also revealed that HIV infection linked significantly to ESBL-producing *KP* and a higher risk of mortality.

There is limited information on the association of HIV infection and Gram-negative BSIs. In a Kenyan prospective paediatric study, *KP* ranked second behind *Escherichia coli* in the aetiology of nosocomial Gram-negative BSIs, causing 20% (43/156) of the BSI. Healthcare providers performed HIV testing in 27% of admissions, with 2% known to be positive. Overall, the case-fatality rate of nosocomial BSI was 53% compared with 24% in children with community-acquired BSI. The data did not reveal the contribution of HIV infection to mortality (Aiken et al., 2011). HIV-infected African children harbour an array of colonising potential pathogens including ESBL-producing *Enterobacteriaceae* (Cotton at al., 2008); this places them at risk for invasive infections. The South African government introduced a national rollout of ART for South African HIV-infected children in 2004, with access progressively improving throughout the study period. The present study findings should spur continued advocacy for the elimination of paediatric HIV disease in sub-Saharan Africa and the continued comprehensive management of all HIV-infected children.

6.6 Other key findings

Based on the literature review, this study is probably the first comprehensive analysis of *Klebsiella pneumoniae* BSIs (KPBSI) in hospitalised children at a dedicated children's hospital in sub-Saharan Africa. The study revealed that extended-spectrum-beta-lactamase-producing *KP* was an important cause of healthcare-related BSI at this children's hospital. This type of infection was associated with high mortality.

In a 5-year analysis of routine blood culture results from RCWMCH, 56.6% (1679/2969) of all pathogenic isolates cultured were Gram-negative organisms. *KP* (41.8%) and *Escherichia* (29.1%) were the dominant *Enterobacteriaceae* isolates. The proportion of *Enterobacteriaceae* infections *KP* caused declined significantly from 45.8% in 2008 to 31.7% in 2012 (Lochan et al., 2013). These findings are consistent with the present study where the annual incidence rate of KPBSI increased from 2.32 cases per 1000 hospital admissions per year in 2007 to 3.75 in 2009. This then declined to 2.25 in 2011.

The incidence rates of KP BSIs at RCWMCH have undergone reductions overall with a curious surge over 2009 and 2010. The following postulate may have some bearing: the South African measles epidemic started in the Gauteng province, spread to the Western Cape Province of South Africa in 2009, peaked during the diarrhoeal surge season in March and April 2010, and abated in 2011. Of 18 431 national laboratory-confirmed measles cases, infants were the most severely affected both in terms of requiring hospitalisation and in terms of death; HIV-infected children bore the brunt of mortality (Ntshoe et al., 2013; le Roux et al., 2012). At the same time, pandemic *Influenza A* H1N1 swept through the country as part of the 2009 global pandemic. Both measles and *Influenza* A *H1N1* cause severe respiratory dysfunction as well as acute gastroenteritis. The effect of both illnesses was associated with severe pressure on beds and subsequent overcrowding in all wards of RCWMCH. These epidemics affected every healthcare facility within the province equally. It is possible that viral-induced lung damage together with the pressure on limited resources and a likely breakdown of infection control measures facilitated the spread of all healthcare associated infections including *KP.* Healthcare providers did not provide evidence of laboratory molecular PCR testing or lack thereof for genetic relatedness of the

nosocomial or healthcare associated isolates. These diagnostic tests are not routine at the institution's NHLS laboratory.

6.7 Study limitations

This hospital-based retrospective study had many of the limitations of a retrospective review. Sedgwick discussed the merits and shortcomings of retrospective studies (Sedgwick, 2014).

This was a tertiary-hospital-based study. The true burden of community-acquired KP BSI may have been underestimated as the other provincial tertiary children's hospital, secondary, or even district hospitals could have admitted and successfully managed these children. An estimate of the true burden would require a thorough review of the NHLS laboratory databases and collaboration with these paediatric wards outside of RCWMCH.

Hand written patient records provided much of the patient demographic data during the study period; this was the norm at RCWMCH. The study children were a heterogeneous group of hospitalised infants and children with diverse reasons for hospitalisation cared for in different wards throughout the hospital. The recorded detail of their clinical progress and temporal sequence of events varied somewhat in the different disciplines. For instance, the nursing and clinician notes for children within the PICU contained considerably more detail as compared to the notes for children in other wards. There was also clinician-to-clinician variation in the medical wards as well as in the case of medical notes versus surgical notes. Further, as the study was retrospective, it was difficult to determine accurately the exact clinical events or even grand round discussions healthcare providers did not record in the case notes that triggered the investigations. One example was the fact that not all

study children experienced fever at the time of the KP BSI. Horan et al. advocate that healthcare providers may consider and report clinical sepsis for CDC surveillance purposes in neonates and infants in the absence of fever (Horan et al., 2008). The exact timing of the blood cultures at the bedside was almost never recorded in the case notes. However, recording at the NHLS laboratory was documented accurately, but there may have been delays from drawing of blood culture to receipt at the laboratory. The same is true of the exact timing of empiric antibiotic administration. This created difficulties in the analysis of treatment delays. In addition, the case notes did not contain records of the volumes of blood drawn for blood culture. They almost certainly did not conform to consensus guidelines that RCWMCH introduced in 2014 (Lochan et al., 2014). As such, using very low volumes of blood, less than one millilitre as was popularly practised in some cases, may have missed some children with KP BSI. This would lead to underestimating the case numbers in both the community-acquired group as well as the healthcare-related group.

WHO Integrated Management of Childhood Illness (IMCI) guidelines indicate that healthcare providers should consider infants and children less than 5 years old who have possible serious infections at primary care level and as such should give intramuscular ceftriaxone prior to transfer to their referral centre (DOH SA, WHO, UNICEF, 2014). This could also have lead to underestimating at least the non-ESBL KP BSI cases.

The laboratory dataset was incomplete as sensitivities to piptazobactam were not available for all isolates, the clinical dataset was also incompletete as individual records were missing in some cases. Furthermore, inaccurate note keeping made it difficult to verify clinical data or decisions made during grand rounds. Relevant risk

factor details may have varied considerably in the records, which is another problem with retrospective data collections.

The definitions used to classify the type of infection into nosocomial, healthcare-associated, and community-acquired are not the current CDC classifications but an older version of what the CDC has cited. Horan et al. reported the CDC's updated definitions replaced the term *nosocomial infection* with the term *healthcare-associated infection* (HAI) and recognised that these HAIs only include those that were not present on admission (Horan et al., 2008). Further, the 2013 CDC Protocol Clarifications no longer refer to *community-acquired infections* but rather to *present on admission* (POA) infections such as documented signs and symptoms of infection present prior to admission and during the first 48 hours of admission (CDC, 2013). According to these definitions, there is no separation between patients residing in intermediate healthcare facilities and high-risk individuals harbouring resistant hospital-related organisms. Thus, the new definitions would only consider them as having POA infections or HAI.

There has been much said about inconsistencies of such classifications in the literature. This includes recognizing that children residing in intermediate healthcare facilities require a different definition of BSI acquired within those facilities and have infections related to their previous healthcare contact (Henderson et al., 2013). The definitions chosen in this study should provide clarity to understanding KP infections in children residing in intermediate facilities, a subset belonging to HAIs. This allowed an exploration of the antibiotic profile of the *KP* isolates to see whether BSI infections in these children with these specific HRF need different antibiotic cover. The study results indicated that the susceptibility patterns of the KP bloodstream isolates of this subgroup of children suggest that healthcare providers should regard them as

nosocomial. In this study, a created definition combined children with nosocomial KP BSI with those with healthcare-associated KP BSI into one group called healthcare-related KP BSI. Healthcare researchers await further clarity in consensus definitions. However, the outcomes of KP BSIs in children living in intermediate facilities are not different to children living at home, and they require the same effective therapy. Although the study was also limited by the fact that it addressed a single organism, the study allowed comparison with a parallel study on *Staphylococcus aureus* BSI at this same study site (Naidoo et al., 2013). Together the studies provide clinically relevant information for the appropriate antibiotic treatment of *KP* and *Staphylococcal aureus* BSIs at RCWMCH.

The presence of septic shock was a clinical decision made at the discretion of the attending physicians and helped to define the binary for the presence or absence of septic shock. Whether these decisions conformed to consensus definitions could not be determined in this retrospective study. Further, rectal swabs were not routine in children re-admitted within a defined time. As a result, one was unable to use the study to speculate further on the role of shock and gut pathogen colonisation in both ESBL acquisition and risk of mortality.

Skin excoriation relied on nurses or doctors' documentation in their notes rather than being defined by a consensus definition. Nevertheless, it is possible that in some cases the condition was present but not documented in the case notes; additionally, the extent and severity could not be standardised. The terminology used to describe the dermatological changes did not follow consensus definitions and could not be verified retrospectively. At least one such document exists for the description of skin changes and failure in dying patients (Sibbald et al., 2009).

What the admitting doctors and any pharmacy prescription sheets available documented limited the history and detail of exposure to previous hospitalisation and antibiotics. Due to the retrospective design of the study, the study did not capture any outpatient exposures and admissions to hospitals other than RCWMCH that the RCWMCH folders did not record.

Despite the established benefit of early ARV therapy in children identified as HIV infected and the ready availability of HIV testing at RCWMCH, 122 children had unknown HIV statuses. This may have limited fully understanding the effect HIV infection had in the epidemiology of *KP* BSI. This study was also limited to the epidemiology of only one member of the *Enterobacteriaceae* family: *KP*. As such, the bigger picture and the roles of the other Gram-negatives in the aetiology of paediatric healthcare associated infections remain unclear. Expanding on this and including other healthcare-related bacterial pathogens with the knowledge of their resistance patterns would do much to enhance management strategies. This knowledge and ranking, together with the clinical detail, would have significant clinical implication. The complexity and severity of illnesses that force some children to remain within the healthcare environment for prolonged periods may lead to a proportion inevitably succumbing to healthcare-related infections. The outcomes of these children depend somewhat on the correct treatment choices.

The risk factors of exposure to ESBL-related BSI as well as those relating to mortality found in the study were those of association rather than of proven causation. This is inevitable in a retrospective description. However reproducibility of significance of pre-hospital treatment with 3rd and or 4th generation cephalosporins in other studies suggests that this may be causal.

Finally, there may have been referral bias. The information came from only one of 11 provinces in South Africa: the Western Cape, and from one tertiary hospital. This makes generalizability of results difficult.

6.8 Study strengths

The study is the first large African study addressing a single important Gram-negative organism, *KP*, in a hospital-based epidemiological study. The study helped to identify all children with laboratory confirmed *KP* BSIs at RCWMCH during the study period 1st January 2006- 31st December 2011. Only counting the first episode of BSI in the cases where healthcare providers took more than one blood culture around the time of the bacteraemic event helped to prevent over-representing numbers.

Further exploration of variables identified by univariate analysis in an adjusted analysis helped with identifying associations between ESBL-associated infection and mortality. The factors so identified have good clinical relevance in the management of sick children.

The definitions used in the study in categorising the origins of the KP BSIs have enabled insight into the antibiotic susceptibility patterns of KP infections acquired by children living in intermediate facilities. This should enable appropriate antibiotic prescribing in the future. Gleaning the spectrum of risk factors from published paediatric reports as well as through years of clinical exposure to sick children gave sound clinical pertinence.

The severity of the implications of HAIs have been realised because of this and other analyses at RCWMCH. Therefore, RCWMCH has introduced several specific interventions to reduce the risk of nosocomial infections. Active surveillance of nosocomial infections occurs at laboratory level with positive blood culture results

fed back to clinicians via the Paediatric Infectious Diseases Unit (PIDU). A single part-time nurse whose responsibilities lie in other areas than hospital infection control has run education strategies to reinforce hand washing and wide spread use of appropriate hand sanitizers. Management has made standardised guidelines for the drawing of blood cultures available throughout the hospital. Management has also introduced antibiotic stewardship grand rounds as a necessary strategy not only to curb inappropriate antibiotic prescribing but also to optimise patient outcomes. There has been some commitment by hospital management to reduce overcrowding in all the wards, particularly the short stay wards (SSW), the provision of negative pressure ventilation systems, and an increase in the number of isolation cubicles available during recent upgrades of two of the general medical wards. These steps may well have been partly responsible for the reduction of the incidence of this multi-drug resistant organism at RCWMCH. On-going surveillance and acceptance of recommendations to engage Hospital Managers to set up a dedicated hospital infection control unit should provide further evidence of this speculation.

The study findings may not be generalizable to community settings as the study site was a tertiary-hospital-based review. However, some of the lessons about antibiotic misuse, poor hygiene, and particularly the poor outcome of children with this form of BSI are relevant to all healthcare facilities.

Finally, the study has largely addressed the stated study objectives, including an attempt at addressing the knowledge gap on the lack of African data on an important pathogen.

CHAPTER SEVEN: CONCLUSION

7.1 Conclusions

This study has made an important contribution to the understanding of paediatric KP BSI in hospitalised children. It contains a description of the emerging problem of multi-drug resistant *KP* BSIs at RCWMCH.

Key messages:

- *KP* was an important cause of healthcare-related BSIs in the period 2006-2011.
- ESBL-production by *KP* requires careful attention in the consideration of the empiric antibiotic regimens that healthcare providers draw up for the different illness categories.
- ESBL-production by *KP* significantly diminishes the number of affordable antibiotic choices.
- *KP* BSI was associated with high mortality.
- Infants and HIV-infected children were vulnerable groups in the setting of healthcare infections and had increased mortality.
- The high prevalence of healthcare-related infections has major implications for patient outcome.
- Healthcare providers should regard KP BSI in children living in intermediate healthcare facilities as having the same antibiotic susceptibility patterns as those who develop nosocomial KP BSI.

7.2 Recommendations

- There is a need to investigate KP infections in other parts of South Africa as this would enable healthcare providers to clarify the underlying risk factors which may inform antibiotic and infection control practice.
- The study findings provide support to all efforts aimed at strengthening infection control measures.
- Antibiotic stewardship is a non-negotiable strategy that healthcare providers must accept and is critical at all levels of healthcare facilities. Clear hospital antibiotic guidelines for the use of both first-line and second line antibiotics should be available to all prescribers. This stewardship lies within the collaborative ambit of infectious diseases specialists, pharmacy departments, and microbiology departments.
- Healthcare providers should develop standard operating procedures (SOP) to control and prevent hospital acquired infection at ward level including: sterile insertion of peripheral venous cannulae, including careful site preparation and specified maximum duration of a single peripheral intravenous cannula; how to set up and manage intravenous infusions in a sterile manner and how to manage and use multi-dose intravenous medication vials
- Healthcare providers should pay careful attention to managing areas of broken skin in children.
- HIV-infected children need careful comprehensive management to remain well.
- Infection control measures are key interventions in reducing the risk of healthcare associated infections; therefore, hospitals should commission a dedicated infection control unit.

- Collaborative reviews of BSIs in children within the province would provide meaningful information that could potentially identify targeted interventions.
- Further efforts to reduce the socioeconomic determinants that bring children into healthcare environments must continue if South Africa is to achieve the Millennium Development Goals (MDG) 4 targets.
- A well-designed prospective epidemiological study looking at all the *Enterobacteriaceae* with a detailed analysis of strain identification and rigorous clinical data correlation may provide further insight into a complex but important paediatric problem. This, however, would require considerable resource allocation.

7.3 Summary of what can be done

Many challenges exist in the prevention of nosocomial sepsis including how to reduce overcrowding, enforce infection control measures and strict antibiotic stewardship, and deal with careless antibiotic use by healthcare professionals (Hague, 2011). Healthcare providers should curtail indiscriminate use of 3rd generation cephalosporins and should continue to enforce strict approval protocols for prescribing carbapenem antibiotics. Good hand hygiene practice is a fundamentally simple and effective way to help contain the problem of nosocomial infections even in the face of overcrowding. Cohorting and isolating patients carrying drug resistant organisms would also provide some protection to other hospitalised children against the spread of infection. Another improvement would be improved staff training on the aseptic handling of all procedures pertaining to intravenous infusions. Admission rectal swabs on all children that have been previously hospitalised, up to 12 months, may allow better infection control practices. This public children's hospital needs a regular re-evaluation of the prevalence of nosocomial sepsis after implementing

aggressive preventative interventions. Microbiologic characterisation plays an important role in identifying children at risk and guiding therapy. Improved techniques, if available and affordable, for rapid and accurate identification of resistant isolates are also important.

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APPENDICES

Appendix A: Data collection sheet

Study title: *Klebsiella pneumoniae bloodstream infections in hospitalised children at Red Cross War Memorial Children's Hospital: 2006-2011*

Biographical information

Study number		
Folder number		
Date of birth		
Gender	MALE	FEMALE
Admission mass (kg)		Z-score:

Admission status

Date of admission to hospital	
First ward location	
Initial diagnosis	
Subsequent ward movements before diagnosing *K. pneumoniae* bloodstream infection	
Ward	Date of admission

Past admission history

Admission during preceding 28 days	YES	NO	UNKNOWN
Admission during preceding 1 year	YES	NO	UNKNOWN
Number of admissions in preceding year			
Details of most recent admission			
Date of admission			
Principal diagnosis			
Duration of admission			

Antibiotic exposure during admission within last year

Exposure to 3rd generation cephalosporins	YES	NO
Generic name of drug	Period of exposure(dates)	
Exposure to 2rd generation cephalosporins	YES	NO
Generic name of drug	Period of exposure(dates)	
Exposure to fluoroquinolones	YES	NO
Generic name of drug	Period of exposure (dates):	
Exposure to macrolides	YES	NO
Generic name of drug	Period of exposure (dates):	
Exposure to Cotrimoxazole	YES	NO
Generic name of drug	Period of exposure (dates):	
Exposure to carbapenems	Yes	No
Generic name of drug	Period of exposure (dates):	
Exposure to aminoglycosides	Yes	No
Generic name of drug	Period of exposure (dates):	

Exposure to Piptazobactam	Yes	No
Generic name of drug	Period of exposure (dates):	

HIV infection

Patient tested for HIV		YES	NO	UNKNOWN
HIV Test	Date		Result	
Rapid				
HIV ELISA				
HIV DNA PCR				
HIV status	exposed	infected	uninfected	UNKNOWN
CD4 completed	YES	NO	UNKNOWN	
CD4 date				
CD4 absolute count		CD4 percentage		
On ART at time of infection	YES	NO	UNKNOWN	
Date of starting ART		Time on ART (days/months)		

Potential risk factors

Resident in long term health facility	YES	NO
Permanent indwelling catheters / tracheostomy	YES	NO
Date of Insertion		
Date of removal: (if applicable)		
Current admission (before diagnosing bloodstream infection):-		
Intravenous canula / access	YES	NO
Intraosseous access	YES	NO
Central intravenous catheter	YES	NO
IV fluid administration	YES	NO
Duration of IV fluids before bloodstream infection (days):		
Naso- / Endo-tracheal intubation	YES	NO
Tracheostomy	YES	NO
Urinary Catheter	YES	NO
Surgery during admission	YES	NO
Procedure type		
Excoriated skin	YES	NO
K pneumoniae isolation within last year	YES	NO
Date of *K pneumoniae* isolation		
Other		

Underlying medical condition

Chronic lung disease	YES	NO
Chronic renal failure	YES	NO
Immunosuppressive therapy	YES	NO
Long-term glucocorticosteroid administration (>1mo)	YES	NO
Congenital syndrome (specify)	YES	NO
Other	YES	NO
Specify		

***K pneumoniae* bloodstream infection**

Date of blood culture:	
Classification:-	Nosocomial
Community-acquired	Institution-associated community acquired
Evidence supportive of invasive infection:-	
Fever > 38°C	Clinical diagnosis of invasive infection
Clinical focus of infection	Elevated C-reactive protein
Elevated white cell count	Low white cell count
Other (specify):	
Focus of infection	
Primary bloodstream infection	Catheter-related bloodstream infection
Pneumonia	Urinary tract infection
Meningitis	Soft tissue infection
Septic arthritis	Osteomyelitis
Other (specify):	

Investigations at time or close to bacteraemic event

Full blood count:-		
Haemoglobin	g/dL	
MCV	fL	
White blood count	$x10^9$/L	
Neutrophil count	$x10^9$/L	
Lymphocyte count	$x10^9$/L	
Platelet count	$x10^9$/L	
C-reactive protein / PCT	mg/L	µg/L

Antibiotic sensitivity of cultured organism

Ampicillin	S	I	R	N/S
Cotrimoxazole	S	I	R	N/S
Co-Amoxiclav	S	I	R	N/S
Erythromycin	S	I	R	N/S
Gentamicin	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
Ciprofloxacin	S	I	R	N/S
Piptazobactam	S	I	R	N/S
Imipenem	S	I	R	N/S
Meropenem	S	I	R	N/S
Ertapenem	S	I	R	N/S
Colistin	S	I	R	N/S
Tobramycin	S	I	R	N/S
Chloramphenicol	S	I	R	N/S
Nalidixic acid	S	I	R	N/S
Nitrofurantoin	S	I	R	N/S

Other (specify)	S	I	R	N/S

Antibiotic administration: 1. Antibiotics prior to the blood culture

	Name	Date started	Date ended	Duration	Route
Antibiotic 1					
Antibiotic 2					
Antibiotic 3					
Antibiotic 4					
Antibiotic 5					
Antibiotic 6					
Antibiotic 7					
Antibiotic 8					
Antibiotic 9					
Antibiotic 10					
Antibiotic 11					
Antibiotic 12					

2. Empiric and definitive antibiotics in response to bloodstream infection

Antibiotics given?	YES	NO

	Name	Date started	Date ended	Duration	Route
Antibiotic 1					
Antibiotic 2					
Antibiotic 3					
Antibiotic 4					

Complications and outcome

Complication associated with bloodstream infection:-			
Septic shock		DIC	
Respiratory failure		Renal failure	
Hepatic dysfunction		Other (specify):	
ICU admission required	Yes	No	Patient In ICU
Indication for ICU admission:			
Total length of hospital admission:			
Hospital outcome	Discharged	Died	
Date of discharge / death:			
Cause of death:			

Appendix B: Ethics approval certificate

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

22 September 2009

REC REF: 367/2009

Prof B Eley
School of Child & Adolescent Health
Red Cross Children's Hospital

Dear Prof Eley

PROJECT TITLE: KLEBSIELLA PNEUMONIAE BACTERAEMIA AT RED CROSS CHILDREN'S HOSPITAL: A THREE YEAR SURVEY.

Thank you for submitting your study to the Research Ethics Committee for review.

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

Approval is granted for one year till the 30th September 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

S Thomas

Appendix B continued: Extension of Ethics approval certificate

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

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CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

S Thomas

Appendix C: Protocol amendment Pg1

UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE
06 AUG 2014
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)			
☑ Approved	☑ Type of review: Expedited	☐ Full committee	
This serves as notification that all changes and documentation described below are approved.			
Signature Chairperson of the HREC	Signature removed	Date	6/8/14

Note: All amendments should include a Synopsis justifying the changes for the amendment (please see notice dated 23 April 2012)

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	24 July 2014		
HREC REF Number	367/2009		
Protocol title	Klebsiella pneumoniae bacteraemia at Red Cross War Memorial Children's Hospital: 2006-2011		
Protocol number (if applicable)			
Principal Investigator	Heloise Buys		
Department / Office Internal Mail Address	Paediatrics and Child Health		
1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)		☐ Major	√ Minor
1.2 Does this protocol receive US Federal funding?		☐ Yes	√ No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval?		☐ Yes	N/A

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval.
This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.

Revised title:

1. Klebsiella pneumoniae bloodstream infections in hospitalised children at Red Cross War Memorial Children's Hospital: 2006-2011
2. "bacteraemia" replaced by "bloodstream infection" throughout the document
3. Removal of Methods item IV

"IV. Molecular characterisation. Pulsed field gel electrophoresis will be carried out on selected isolates to characterise the genetic relatedness of the isolates. This information can then be used in conjunction with the clinical and demographic information to describe the extent of cross-infection in the hospital. It will also be useful to examine the relatedness of any community acquired isolates (particularly antibiotic resistant community acquired isolates) to nosocomial isolates. This may assist in determining risk factors for community acquired acquisition of ESBL-producing K. pneumoniae isolates.

Appendix C: Protocol Amendment Pg2

Preliminary PFGE characterisation of ESBL-producing K. pneumoniae isolates from children recently admitted to the short stay ward showed no relatedness of these isolates, making it unlikely that S11 was the source of the organisms that infected those children. However, knowledge of the genetic background of true nosocomial strains will allow for more extensive evaluation of these results."

- Removed as sample collection did not proceed beyond initial preliminary sampling

4. Revision of case definitions: Under "Healthcare-associated infection: item II:

"II. Hospital onset infection Isolation of Klebsiella pneumoniae on a blood culture taken after 48 hours of admission to hospital, or within 48 hours of discharge from hospital plus one or more risk factors for health care associated infection (refer above)."
i.e.
"Healthcare-associated risk factors (HRFs)

- Presence of an invasive medical device e.g., vascular catheter
- Surgery, hospitalisation, dialysis or residence in an intermediate healthcare facility in the preceding 12months of admission
- History of KP infection or colonization (adapted for this study)"

Appendix D: Annual progress report

UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE
06 AUG 2014
HEALTH SCIENCES FACULTY

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30 DEC 2014.
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signature removed	Date Signed	6/8/2014

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	24.July 2014		
HREC REF Number	367/2009	Current Ethics Approval was granted until	15 December 2013
Protocol title	Klebsiella pneumoniae bacteraemia at Red Cross War Memorial Children's Hospital: 2006-2011		
Principal investigator	Dr Heloise Buys		
Department / Office Internal Mail Address	Paediatrics and Child Health		
1.1 Does this protocol receive US Federal funding?		☐ Yes	√ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
√	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		410
Total number of records or specimens collected, reviewed or stored since last progress report		
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☐ Yes	√ No

4. Signature

Signature of PI	Signature removed	Date	4/8/2014
Signature of Supervisor (if PI is a student)	Signature removed	Date	