MICROBIOLOGY OF PUERPERAL SEPSIS AND ITS CLINICAL IMPLICATIONS AMONG HIV-INFECTED AND HIV-UNINFECTED WOMEN IN A HOSPITAL SAMPLE IN ZIMBABWE

By
Dr Rumbidzai Majangara

Thesis submitted in partial fulfillment of the degree of Masters in Medicine Obstetrics and Gynaecology, University of Zimbabwe

Supervised by Dr M. F. Gidiri and Professor Z. M. Chirenje

Department of Obstetrics and Gynaecology

College of Health Sciences, University of Zimbabwe

June 2016

ABSTRACT

Title: Microbiology of puerperal sepsis and its clinical implications in a Hospital sample in Zimbabwe

Introduction: Puerperal sepsis is infection of the female genital tract occurring at any time between the rupture of membranes or labour, and the 42nd day postpartum. Puerperal sepsis has become the leading cause of maternal death in Harare public health institutions accounting for 19% and 30% of maternal deaths for the years 2010 and 2014 respectively, from being the fourth nationwide cause at 12.3% in the year 2007. The objectives of this study were to determine the identity and antibacterial susceptibility profiles of bacteria colonizing the genital tract and blood stream, and to assess clinical outcomes and association with HIV infection in women with puerperal sepsis.

Methodology: A prospective cohort study was conducted at Parirenyatwa and Harare Hospitals between 02 September 2014 and 01 July 2015. Endocervical swabs and blood were collected for culture and susceptibility testing from 151 consecutive women who met the World Health Organisation criteria for puerperal sepsis. HIV sero-status, immunological status and antiretroviral therapy (ART) use were determined. Medical records were reviewed for assessment of clinical outcomes. Proportions, categorical values and means were compared using Z-test, χ^2 test and t- test along with 95% confidence interval (CI) and p-value of <0.05.

Results: The mean age was 25.1 ±5.8years and most women were multiparous (53.6%). The majority of women had delivered at a hospital (78.1%) and by caesarean section (57.6%). The commonest bacterial isolates were *Escherichia coli* (30.6%) and *Klebsiella pneumoniae* (15.3%). Multidrug resistant organisms (MDRO) accounted for 10.9% of the isolates. MDRO were associated with prolonged mean hospital stay 23.0days (d) compared to 10.5d in women without MDRO (p=0.009). The frequency of genital colonization with *Enterobacter species* was significantly higher in HIV infected (9.1%) than uninfected women (1.7%) (p=0.04). Among HIV infected women (21.9%), severe immunosuppression (CD4 $<200/mm^3$) was associated with a greater need for laparotomy 42.9% vs 4.5% (p=0.01) and prolonged mean hospital stay 19.0d vs 10.2d (p=0.03) compared to mild-advanced (CD4 count 200-500/ mm^3) and insignificant immunosuppression (CD4 $>500/mm^3$). There was a non-significant trend towards, earlier onset of sepsis; and higher rates of pelvic abscess, septic shock, wound dehiscence, peritonitis, death and need for admission into the intensive care unit (ICU) in women with severe immunosuppression. Antiretroviral therapy use did not independently influence outcomes. Puerperal sepsis case fatality rate was 7.3%.

Conclusion: Gram negative bacilli, particularly *E. coli,* are the commonest bacterial isolates in puerperal sepsis. There is emergence of MDRO gram negative bacilli resistant to carbapenems, especially *K. pneumoniae*. MDRO and HIV associated severe immunosuppression are risk factors for prolonged hospital stay and need for surgery. Robust infection control strategies, emphasis on rational drug use and clinical culture surveillance to identify MDRO and monitor epidemiologic trends is recommended.

ACKNOWLEDGEMENTS

I am grateful to the following:

- My supervisors Dr M F Gidiri and Professor Z M Chirenje for the professional assistance in designing and overseeing the completion of this study
- Professor V Robertson for professional advice on microbiological laboratory considerations
- NECTAR Mentored Research Scholarship Program for financial assistance and education on principles of research
- University of Zimbabwe (UZ) Department of Microbiology Laboratory Scientists Ms C Berejena, Ms T Magombei and Mr R. Mudengezerwa for assistance in carrying out microbiological laboratory procedures
- Mr M Munjoma UZ Obstetrics and Gynaecology laboratory scientist for professional advice
- Staff at Harare Hospital and Parirenyatwa Hospital in the following departments- Maternity, Gynaecology, Casualty, Intensive care and Outpatient units for assistance in notification of patients; and the Public Health Laboratory for provision of results for tests performed as standard of care
- Women who agreed to participate in the study
- Mr K Chimunda for assistance in statistical analysis of study results

Contents **Page number**

LIST OF TABLES, FIGURES AND APPENDICES

Tables:

Figures:

Appendices:

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency Syndrome
API	Analytical profile index
ART	Antiretroviral therapy
ATCC	American Type Culture Collection
BA	blood agar
bd	twice daily
BMI	body mass index
CA	chocolate agar
CD4	cluster of differentiation 4
CI	95% Confidence Interval
CO_2	carbon dioxide
CoNS	coagulase negative staphylococcus
dl	deciliter
EDLIZ	Essential Drugs List and Standard Treatment Guidelines for Zimbabwe
FBC	Full blood count
GAS	Group A streptococcus
g/L	grams per liter
g/dl	grams per deciliter
h	hour
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
IM	intramuscular
IQR	interquartile range
IV	intravenous
kg	kilogram

MAC	MacConkey
MDRO	multidrug resistant organisms
Mcg	micrograms
mg	milligrams
ml	milliliter
ml/kg/h	milliliters per kilogram per hour
mm^3	cubic millimeter
MU	Mega Units
PMTCT	Prevention of mother to child transmission of HIV
po	orally
qid	four times daily
RR	Relative Risk
sd	standard deviation
sp	species
Stat	immediately
tds	three times daily
UK	United Kingdom
USA	United States of America
U&E	urea and electrolytes
UZ	University of Zimbabwe
WHO	World Health Organisation

CHAPTER 1

1.0 INTRODUCTION

Puerperal sepsis is infection of the female genital tract occurring at any time between the rupture of membranes or labour, and the 42nd day postpartum in which 2 or more of the following are present: pelvic pain, fever (i.e. oral temperature 38.5°C or higher on any occasion), abnormal vaginal discharge (e.g. presence of pus), abnormal smell/foul odour of discharge, and delay in the rate of reduction of the size of the uterus (<2cm/day during the first 8 days) (1,2).

Globally, the incidence of puerperal sepsis ranges from 2.7% to 5.7% (1). Puerperal sepsis accounts for 2% of maternal deaths in the developed world and 10-12% of maternal deaths in the developing world(3). Puerperal sepsis was the fourth leading cause of maternal death in Zimbabwe accounting for 12.3% of maternal deaths in a national survey in the year 2007(4) . An unpublished retrospective review comparing trends in maternal mortality for the public maternity institutions of Harare reported that obstetric sepsis was now the leading cause of maternal deaths accounting for 19% and 30% of maternal deaths for the years 2010 and 2014 respectively (5). In comparison, the contribution of genital tract sepsis to maternal death may not be as significant in the developed world. Though direct and indirect sepsis combined were the second commonest cause of maternal deaths in the latest United Kingdom Triennial Report for 2011-2013, genital tract sepsis was the fourth leading cause of direct maternal death accounting for 0.29 maternal deaths per 100 000 maternities, and was in fact showing a downward trend from preceding years (6).

If left untreated women may develop early complications such as wound dehiscence, peritonitis, pelvic abscess, necrotizing fasciitis, toxic shock syndrome; and long-term maternal complications such as chronic pelvic inflammatory disease, chronic pelvic pain and infertility(2,7). The proportion of women developing infertility following puerperal sepsis ranges from 5% in developed countries to 12% in developing countries(1)**.**

Major risk factors for puerperal sepsis are caesarian section, prolonged and premature rupture of membranes, prolonged labor (>24hours), frequent unsanitary vaginal examinations, retained products of conception, hemorrhage, anemia, malnutrition and obesity (2,7,8). The incidence of puerperal sepsis has been shown to be higher in HIV positive persons compared to HIV negative persons (9,10).

Most pelvic infections are caused by overgrowth of bacteria indigenous to the female genital tract, notably Groups A and B beta-hemolytic streptococci, *Staphylococcus aureus*, enteric bacteria such as *Escherichia Coli*, Klebsiella species, pseudomonas species, clostridium species, *Morganella morganii* and other anaerobes, mycoplasma, chlamydia and gonococci. Infection is frequently polymicrobial(2,7,12). In the developed world, the most virulent infective organism causing life-threatening infection is community acquired Group A beta-hemolytic streptococci (7,13). Studies in developing countries have noted inter-country variance in the most frequent etiologic organisms (14–18).

Antibiotic resistance is a global public health problem worse in developing countries which have a high burden of infectious disease, constraints on microbiological investigations to identify resistant infections and unavailability of novel agents to treat them. There are accelerating rates of antimicrobial resistance fueled by irrational drug use and shortfalls in infection control and public health (19). Treatment of most infections in Zimbabwe is empiric/syndromic mainly due to lack of microbiological services and diagnostic capacity from grassroots up to tertiary level(20). There is therefore a need for periodic evaluation of antimicrobial effectiveness against infective bacteria. This will allow identification of multi-drug resistant organisms (MDRO), and pave way for concerted efforts at preventing their emergence and spread.

1.1 JUSTIFICATION FOR THE STUDY

Most data on the microbiology of puerperal sepsis in Zimbabwe was collected over 2 decades ago before the advent of the HIV epidemic (21,22). Recent estimates of puerperal sepsis in our hospitals come from retrospective studies of maternal deaths, without microbiological investigation (4,5). Therefore these data reflect only clinically defined puerperal sepsis.

In our Zimbabwean institutions, puerperal sepsis is diagnosed clinically as infection of the female genital tract occurring at any time between the rupture of membranes or labour, and the 42nd day postpartum in which 2 or more of the following are present: pelvic pain or tenderness, fever (i.e. oral temperature 38.5°C or higher on any occasion), abnormal vaginal discharge (e.g. presence of pus, abnormal uterine bleeding), abnormal smell/foul odour of discharge, and delay in the rate of reduction of the size of the uterus (<2cm/day during the first 8 days) (1,23).

Empiric treatment is recommended using ampicillin 1-2g IV qid and metronidazole 500mg IV tds and gentamycin 3-5mg/kg IV daily. Ceftriaxone 1-2g IV bd or benzyl penicillin 5MU IV qid are alternatives to ampicillin. Recommended antibiotic prophylaxis before caesarian section is ceftriaxone 1g IV stat or ampicillin 1g IV stat (23). The persistent use of select antibiotic classes leads to development of resistance to those classes over time and may eventually lead to the selection of multidrug-resistant organisms. During the economic crisis of the last decade, there has been a lack of systematic, well-designed and funded research studies to determine the etiology of puerperal sepsis and the emergence of drug resistance. This means that the recommended drug regimens for prophylaxis and treatment of infections might be ineffective in our setting.

HIV infection has been associated with more severe clinical manifestations in most studies of pelvic inflammatory disease, while other investigators found no increase in severity of clinical outcomes (24–27). While evidence exists that HIV infection increases the risk of postpartum infections (9), there is insufficient information on the association of HIV infection with clinical outcomes.

This study aims to identify suspected infecting organisms, assess susceptibility of these organisms to the current recommended empiric antibiotics, and assess the association of these organisms and HIV infection with clinical outcomes in our population.

CHAPTER 2

2.1 LITERATURE REVIEW

Puerperal sepsis is infection of the female genital tract occurring at any time between the rupture of membranes or labour, and the 42nd day postpartum in which 2 or more of the following are present: pelvic pain, fever (i.e. oral temperature 38.5°C or higher on any occasion), abnormal vaginal discharge (e.g. presence of pus), abnormal smell/foul odour of discharge, and delay in the rate of reduction of the size of the uterus (<2cm/day during the first 8 days).Clinical signs and symptoms typically present more than 24 hours after delivery unless the patient had prolonged rupture of membranes or prolonged labour without prophylactic antibiotics when the disease may be noted earlier(1).

Puerperal sepsis can be caused by both exogenous and endogenous bacteria. Endogenous bacteria are usually commensals in the vagina and rectum which may go up the uterus during prolonged rupture of membranes, genital tract trauma or via examining fingers and instruments. Exogenous bacteria may be introduced by unsterile examination, droplet infection, sexual activity and foreign substances introduced into the vagina e.g.- herbs, oils and cloths (1,7) . Puerperal infection following vaginal delivery primarily involves the placental implantation site and the decidua and adjacent myometrium, while the pathogenesis of uterine infection following cesarean delivery is that of an infected surgical incision (7).

Community acquired Group A Streptococcus (GAS) from the woman's own respiratory tract or hands, or hospital staffs' hands, is increasingly causing invasive and life threatening puerperal

infections worldwide (7,28). Puerperal sepsis mostly associated with GAS was the commonest cause of direct maternal deaths in the United Kingdom (UK) during the triennium 2006-2008 (29).

The practice of endometrial aspirate and blood cultures in patients suspected of having puerperal endometritis effectively contributes to the diagnosis and treatment of this infection (30). Studies utilising endocervical swabs have shown an approximately 80% chance of identifying suspected infecting organisms compared to blood cultures which were positive in 5% to 24% of samples from women with puerperal sepsis (7,14,16,17). However genital tract swabs have the disadvantage of picking up other non-infecting vaginal flora (7). In a Nigerian study in which endocervical swabs were cultured in 139 women with puerperal sepsis, *S. aureus*, *E. coli*, *Streptococcus sp.* and *Pseudomonas sp.* were the commonest isolated organisms. There was either insignificant or no growth in 30/139 specimens (18). A study in India in which endocervical swabs were analysed by microscopy, culture and sensitivity, the most commonly isolated organisms were Klebsiellae, *S. aureus*, *E. coli*, *Pseudomonas sp*. and B-hemolytic streptococci. There was no growth in 2/12 specimens (14). In a Bangladesh Study, there was significant growth in anaerobic cultures of 42/50 endocervical swabs collected from women with puerperal sepsis (16). Microscopy and culture of endocervical swabs and blood may identify infecting organisms and assist in the choice of antimicrobials.

Studies including vaginal and caesarian deliveries indicated that HIV-infected women had over three times the risk of a puerperal sepsis compared with uninfected women; this figure increased to nearly six amongst studies only including women who delivered by caesarian section (9). HIV-positive women had a statistically significant increase in the incidence of post-operative fever (RR 1.3) and wound sepsis/sinus (10). In one study, post-partum endometritis was more prevalent in HIV-infected women compared to HIV-uninfected controls (10.3% vs 4.2%), being inversely related to CD4 percentage (31).

The empiric antibiotic regimen for treatment of puerperal sepsis in Zimbabwe is ampicillin 1-2g IV qid, metronidazole 500mg IV tds and gentamycin 3-5mg/kg IV daily. Alternatives to ampicillin are ceftriaxone 1-2g IV bd or benzyl penicillin 5MU qid IV. Alternative to gentamycin in case of abnormal kidney function is chloramphenicol 50mg/kg in 4 divided doses(23). For the management of severe sepsis, a combination of peperacillin/tazobactam or clindamycin and a carbapenem may provide the broadest range.(12). Amoxicillin, ciprofloxacin and erythromycin may be given to complete the antibiotic course on an outpatient basis. Recommended antibiotic prophylaxis before caesarian section is ceftriaxone 1g IV stat or ampicillin 1g IV stat (23).

Persistent use of a select antibiotic class leads to development of resistance to that class over time. Combination empiric treatment may eventually lead to the selection of multidrug resistant organisms. Multidrug resistant organisms (MDRO) are defined as organisms with acquired non-susceptibility to at least one agent in three or more antimicrobial categories (32). Emergence of resistance to multiple antimicrobial agents in both gram negative and gram positive bacteria is a significant threat to public health as there may be no effective antimicrobial agents to manage these

infections, especially in resource limited settings which have limited access to novel antimicrobials. MDRO associated infections are associated with poor patient outcomes (30). Notable MDROs include multidrug-resistant carbapenemase-producing *K. pneumoniae* and *Acinetobacter sp* which are resistant to all currently available antimicrobials or remain susceptible only to older, toxic agents such as the polymyxins (32–35).

Earlier research conducted in Zimbabwe has shown emergence of anti-microbial resistance in pathogens such as staphylococci, streptococci, gonococci and enteric bacteria. A study on antimicrobial susceptibility of *Neisseria gonorrhea* showed >60% resistance to penicillin, >10% resistance to cefuroxime, 50% resistance to erythromycin, 20% resistance to tetracycline and full susceptibility to kanamycin and spectinomycin (36). A study on vaginal pathogens in which 130 isolates of Group B streptococci were isolated showed 5 isolates resistant to erythromycin, 7 isolates resistant to clindamycin, intermediate resistance to gentamycin, while all were susceptible to beta lactams(22). A project investigating the role of *S. aureus* in local burn centers determined a high prevalence of methicillin resistant *S. aureus* (MRSA) (64% and 49% prevalence at Parirenyatwa and Harare Hospital, respectively) among randomly sampled wound cultures (37).

2.2 Research Question

What is the microbiology of the female genital tract and blood stream in HIV infected and HIV-uninfected women with puerperal sepsis, the in vitro antibacterial susceptibility of these organisms, and the clinical response to empiric antibiotics in women admitted at Parirenyatwa and Harare Hospitals?

2.3 Study Objectives

2.3.1 Broad Objective

To determine the microbiology of puerperal sepsis and its clinical implications among HIV-infected and HIV-uninfected women admitted at Parirenyatwa and Harare Hospitals

2.3.2 Specific Primary Objectives

i. To identify the bacteria colonizing the female genital tract in women with puerperal sepsis
ii. To identify bacteria causing bacteremia in women with puerperal sepsis
iii. To determine the antibacterial susceptibility profiles of the cultured bacteria
iv. To determine if the spectrum of microbiological organisms is different among HIV-infected and HIV-uninfected women
v. To compare women's clinical response after empiric antibiotics to in vitro antibacterial susceptibility profiles of colonizing bacteria
vi. To determine proportion of women who develop complications and those who require surgery such as laparotomy for management of complications

2.3.3 Secondary Objectives:

i. To determine risk factors for bacteremia

ii. To determine if immunological status (CD4 cell count), virological status (viral load) and use of antiretroviral therapy are related to presentation of puerperal sepsis

CHAPTER 3

3.0 RESEARCH METHODOLOGY

3.1 Study design

A Prospective Cohort Study

3.2 Study Setting

The study was conducted at Parirenyatwa Hospital and Harare Hospital maternity, gynecological and intensive care units

3.3 Study Population

All women admitted with a clinical diagnosis of puerperal sepsis at Parirenyatwa and Harare Hospitals.

3.4 Inclusion Criteria

- Clinical diagnosis of puerperal sepsis defined as: Infection of the female genital tract occurring at any time between the rupture of membranes or labor and the 42nd day postpartum in which 2 or more of the following criteria are present:
    - Pelvic pain
    - Fever (i.e.- oral temperature 38.5°C/ axillary temperature 38°C or higher on any occasion)
    - Abnormal vaginal discharge (e.g.- presence of pus)
    - Abnormal smell/foul odor of discharge

- Delay in rate of reduction of the size of the uterus(<2cm/day during the first 8 days)

- Able and willing to give informed consent/ proxy available to give informed consent

3.5 Exclusion Criteria

- Unwilling to give informed consent
- Isolated extra-genital infection e.g.– isolated urinary tract infection, chest infection, isolated wound infection

3.6 Sample Size Determination and Sampling Plan

3.6.1 Sampling:

Convenient sampling of all women presenting with puerperal sepsis who met the inclusion criteria

Sample size calculation:

Basing on a blood culture positive (bacteremia) rate of 10% [range 5-20% (8)] among people diagnosed with puerperal sepsis and using the Dobson formula, the sample size needed to give an 80% power to determine the bacteriology of puerperal sepsis and its clinical implications in patients admitted at Parirenyatwa and Harare Hospitals is 138.2976. Incorporating a 10% loss to follow up rate, 151 participants were needed.

VIZ:

$$n = \frac{Z^2_{\alpha/2}\, P(1-P)}{\Delta^2}$$

Where:

$Z^2_{\alpha/2} = (1.96)^2$

P= proportion with positive blood culture = 0.1

$\Delta^2 = (0.05)^2$

3.7 METHOD OF DATA COLLECTION

3.7.1 Face to face interviews and clinical examinations were conducted to assess inclusion criteria and acquire informed consent from each participant. Height and weight were measured on admission.

3.7.2 An interviewer administered questionnaire was used to collect information on:

- Demographic data such as age of patient and partner, physical address, level of education, employment status, parity and marital status.

- Index pregnancy factors (factors associated with the last pregnancy) such as booking status, number of fetuses, gestational age at delivery, place of delivery, mode of delivery, induction of labor, pre-labor rupture of membranes, duration of labor, meconium staining of liquor, method of placental delivery, perineal/genital tract trauma, postpartum hemorrhage. Medical records from antenatal care and delivery were used to verify information.

Definitions:

- Booking was assessed according to the World Health Organization recommendation of 4 focused antenatal care visits. Women were classified into those who attended ≤3 antenatal care visits and those with ≥4 antenatal care visits (38)**.**
- Prolonged labor was labor of duration greater than 24 hours (39)
- Postpartum hemorrhage was blood loss >500ml at delivery (40)
- Weight and height were measured on admission. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in m^2. Women were classified as underweight (BMI<18.50kg/m^2), normal weight (BMI18.51-24.99 kg/m^2), overweight (BMI 25.00-29.99 kg/m^2), obese (BMI ≥ 30.00 kg/m^2) (41)
- Preterm delivery was birth after 28weeks 0days but before 37 weeks 0days of gestation (23)

3.7.3 Laboratory Procedures:

3.7.3.1 Blood for culture, full blood count (FBC), urea and electrolytes (U&E) and HIV testing was collected at the time of admission. Where possible, the aim was to collect specimens before administration of antibiotics if this would not delay antibiotic administration by greater than 45 minutes (42).

3.7.3.1.a

Blood culture specimen collection- Blood for culture was collected as below, at the time of admission and tested at the University of Zimbabwe Medical Microbiology Laboratory.

Skin Preparation: A tourniquet was applied and the vein located, then the site of venepuncture was cleansed with 70% alcohol for 30 seconds. This swab was discarded then the site was cleansed with a second swab with iodine solution or alcohol again. The site was allowed to air dry for 1 minute before venepuncture. The vein was not re-palpated.

Bottle Preparation: The bottle surface, the media, and the sensor/autoclave tape were inspected to ensure that the broth was clear and the sensor/autoclave tape was intact. The bottle was not used if the sensor/autoclave tape was not intact, media was cloudy, or if the bottle was cracked or had been dropped. The sterile sensor/autoclave tape was removed; the rubber stopper cleansed with 70% alcohol or iodine solution and allowed to dry for 1 minute before inoculation.

Fourteen milliliters of blood were collected [5ml for blood culture (minimum 5ml), and 4ml for FBC, 4ml for U&E, 1ml for HIV]. The blood was inoculated first into the culture bottle containing 50ml of 30g/L tryptone soya broth before other blood collection tubes. The specimen and case investigation forms were labeled and sent to the laboratory for immediate incubation.

3.7.3.1.b Full blood count, urea and electrolytes were tested at the public health laboratories as per standard of care and results collected from verifiable source documentation. Anemia was defined as a hemoglobin <10.0g/dl (43)**.** Renal failure was defined as an increase in serum creatinine by ≥26.5 micromoles/L within 48 hours; or ≥50% increase in serum creatinine within the prior seven days; or urine volume <0.5 ml/kg/h for six hours(44)

3.7.3.1.c The HIV result was obtained from a primary source document if it was performed within 3 months of presentation. If there was no documented HIV test result, provider initiated counseling and testing was offered as per the standard of care. The CD4 count result was also collected from a primary source document if it was performed within 6 months of presentation; otherwise a new specimen was collected and tested. Women were designated an immunological class based on their CD4 count: Insignificant immunosuppression if CD4 count >500/mm³, mild immunosuppression if CD4 count 350-499/mm³, advanced immunosuppression if CD4 count 200−349/mm³, and severe immunosuppression if CD4 count <200/mm³ (45)**.** HIV viral load was not tested for any of the HIV infected women due to funding constraints.

3.7.3.2 Endocervical swabs for culture and sensitivity were collected as below, on admission and tested at The University of Zimbabwe Medical Microbiology Laboratory.

Endocervical specimen collection – An unlubricated sterile Cusco's speculum was passed into the vagina to visualize the cervix. A sterile dry cotton-tipped swab was then inserted into the endocervix and rotated for 15-30seconds to collect lochia, avoiding contact with vaginal walls both at entry and removal of the swab. The swab was immediately placed into a tube with Amies transport media. The specimen and case investigation forms were labeled and transported to the laboratory within 24 hours of collection at 4-8^0C in a specimen carrier with frozen ice packs or at room temperature if ice packs were unavailable.

3.7.3.3 Intra-operative pus swabs were collected for patients who had laparotomy for pelvic abscess by the responsible teams, and tested at the public health laboratory as per standard of care. Isolates were not identified to genus and species level at the public health laboratory, therefore the results were not analyzed.

3.7.3.4 Laboratory handling of specimens- The specimens were processed within 30 minutes of receipt in the laboratory.

3.7.3.4.a Blood culture processing

- The information on the specimen and case investigation form was crosschecked to ensure that it tallied.
- A laboratory reference number was assigned to the specimen and entered into the record book and onto the blood culture bottle.
- The date of laboratory receipt of the specimen was entered into the record book.
- The inoculated blood culture bottle was incubated at 37^{o}C overnight.

- On day 1, blood was sub-cultured on blood agar (BA), chocolate agar (CA) and MacConkey (MAC) plates using an isolation technique then incubated aerobically, but with CA in CO_2, for 24 hours.
- If there was no growth, incubation was continued, and then subculture repeated on day 5.
- If there was no growth on day 5, blood was re-incubated and the subculture repeated on day 10. If there was no growth on day 10, the culture was regarded as negative.
- The bacterial isolates were identified and antimicrobial susceptibility testing performed. See procedure in sections **3.7.3.4.c and 3.7.3.4.d** below.
- The results were documented in the record book and on the case investigation form.

3.7.3.4.b Endocervical swab processing in the laboratory

- Information on the specimen and case investigation form was crosschecked to ensure that it tallied.
- A laboratory reference number was assigned to the specimen and entered into the record book and on culture plates.
- The date of laboratory receipt of the specimen was recorded onto culture plates and record book
- The swab was inoculated sequentially onto 2 BA plates, 1 CA plate and 1 MAC plate, using the isolation technique. 1 BA plate was for aerobic culture and 1 for anaerobic culture.
- A smear was prepared from the swab, gram stained and examined under the microscope to check for organisms in case growth was inhibited on the culture plates.
- The CA plate was placed in a jar of 5-10% CO_2, and the anaerobic BA plate was placed in an anaerobic jar.

- All 4 plates were incubated at 37^{o}C aerobically and anaerobically.
- The aerobic BA, CA and MAC plates were examined for bacterial growth at 24 hours. If there was no growth in aerobic BA and MAC plates, the result was reported as 'no growth obtained'. If there was no growth in CA plate, it was re-incubated for a further 24 hours only.
- The anaerobic plate was examined after 48 hours of incubation. If no growth was obtained, the result was recorded as 'no growth obtained'.
- The bacterial isolates were identified and antimicrobial susceptibility testing performed. See procedure in sections **3.7.3.4.c and 3.7.3.4.d** below
- The results were documented in the record book and on the case investigation form

3.7.3.4.c Bacterial Identification: A gram stain was performed on each isolates. Conventional phenotypic bacterial identification tests were set up and interpreted as per District Laboratory Practice in Tropical Countries guidelines(46). Bacteria that were difficult to identifying using conventional methods were identified using the analytical profile index (API= Biomerieux) system.

3.7.3.4.d Antimicrobial susceptibility testing:

Susceptibility testing was done using the Kirby Bauer technique on all bacterial isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines(47). The Essential Guide to Management of Common Obstetrics and Gynecology conditions in Zimbabwe (2012) and Essential Medicines List and Standard Treatment Guidelines for Zimbabwe (2011) were used to determine the choice of antibiotic discs to use for testing (20,23)**.** The following antibiotic discs were used: ceftriaxone 30 micrograms (mcg), penicillin G 10mcg, chloramphenicol 30mcg,

gentamycin 10mcg, clindamycin 2mcg, ciprofloxacin 5mcg and erythromycin 5mcg. The antibiotics discs were controlled using *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Isolates were inoculated onto Mast Muller Hinton Agar, following which drug impregnated discs were placed and incubated for 24 hours at 37^0C. The inhibition diameter/zone was measured to the nearest millimeter. Organisms with acquired non-susceptibility to at least one agent in three or more antimicrobial categories above were designated as multidrug resistant organisms (MDRO) (32). MDROs were tested for susceptibility to carbapenems (meropenem 10mcg and imipenem 10mcg discs). Meropenem is the carbapenem readily available in Zimbabwean public and private health institutions, therefore it was tested first. The first 3 MDRO (*E. coli, Klebsiella oxytoca, Enterobacter sp)* were susceptible to meropenem therefore imipenem susceptibility was not tested. However, when the fourth MDRO was noted to be resistant to meropenem, susceptibility to both imipenem and meropenem were subsequently tested for the remainder of MDRO. There was no reference for susceptibility tests using the Kirby Bauer technique for the following isolates and respective antibiotics: *Group D streptococcus* (ceftriaxone, gentamycin, clindamycin); *Streptococcus viridans* (penicillin, gentamycin, ciprofloxacin); *Moraxella* (all antibiotics).

Bacillus sp, *Coagulase negative staphylococci* and fungal growth were considered blood culture contaminants in this protocol and antibiotic susceptibility tests were not performed. *Bacillus sp* and *Coagulase negative staphylococci* are part of the indigenous flora of the skin, and their isolation in a blood culture may simply represent contamination. Though both may represent significant growth in special circumstances such as immunosuppression, intravenous drug abuse and indwelling foreign devices; their presence as pathogens should be confirmed by sustained

growth on repeat blood cultures which was not possible in this study (48–50) Bacillus sp are female genital tract commensals therefore antibiotic susceptibility testing was not done if bacillus was isolated from the endocervical swab (7).

3.7.4 Perusal of medical records from local clinics, hospitals and laboratories. These included antenatal care and delivery books/cards, nurses and doctors charts, drug charts and laboratory result slips, to assess:

- Index pregnancy factors
- Clinical examination findings such as blood pressure, pulse, respiratory rate, axillary temperature, abdominal and pelvic examination– to assess clinical response and complications
- Laboratory test results
- Management offered to patient such as type and duration of antibiotic therapy, laparotomy, intensive care unit admission, length of hospital stay, type and duration of antiretroviral therapy

Early retrieval of medical records for extraction into electronic storage was done to minimize loss of data

3.7.5 Responsibility of care for women

Women were admitted and managed as per routine standard of care by the on call physicians. Results of culture and sensitivity testing were availed by the researcher to the attending physicians who utilized them to their discretion. Women were followed up by the researcher until discharge from hospital or death.

3.7.6 **Outcome measures**:

1. Identity of organisms colonizing the female genital tract in puerperal sepsis
2. Identity of organisms causing bacteremia in puerperal sepsis
3. Association of HIV status with microbiology of puerperal sepsis
4. Antibacterial susceptibility profiles of colonizing organism
5. Proportion of women who developed early complications such as wound dehiscence, peritonitis, pelvic abscess, septic shock, renal failure and death
6. Association of antibacterial susceptibility patterns with clinical outcomes such as length of hospital stay, need for ICU admission and proportion of women who developed complications
7. Proportion of women who required surgery e.g.-laparotomy
8. Association of HIV status, CD4 count and use of antiretroviral therapy with time to onset of puerperal sepsis, development of complications, need for ICU admission and length of hospital stay
9. Demographic, obstetric and medical risk factors for bacteremia

3.8.1 Pretesting the Questionnaire

Pre-testing of the questionnaire was done at Parirenyatwa Hospital. Amendments and adjustments were made to the questionnaire where necessary before the final data collection process was done.

3.8.2 Data capture, processing and quality control

Entryware software was used for data entry. Consistency and logic checks were done. Data was cleaned and coded for analysis.

3.9 STATISTICAL ANALYSIS

Data was analysed using Statistical package for Social Scientist (SPSS) version 16. 0. Descriptive statistical analysis was carried out using Z test for proportions, for testing the differences in proportions between the groups. Student‘s t-test for independent groups were used to check relationships on continuous variables while categorical variables were expressed as percentages and frequencies , and compared using the Chi-square test to compute a p-value.. Descriptive statistics were expressed as mean (sd) or median (inter-quartile range [IQR]) for quantitative variables that are normally distributed or skewed respectively. All statistical tests were considered statistically significant if $p< 0.05$.

3.10 ETHICAL CONSIDERATIONS

The study was approved by the Harare Hospital Ethics Committee, Joint Research Ethics Committee and the Medical Research Council of Zimbabwe. The purpose of the study was explained to all potential participants and written informed consent was obtained.

Participants invited to participate in the study were informed that they were free not to participate and that they were free not to answer any questions on the questionnaire and to drop out at any stage of the study and still continue to receive the standard of care equal to research participants.

Strict confidentiality was maintained. Documents with participants' personal information were reviewed by the Principal Investigator and assistants only and kept in a locked cupboard and password locked computer. Personal identifiers were coded with a participant identity number for sensitive tests such as the HIV test. No personal identifiers were used during data analysis and the same will apply for publication.

CHAPTER 4

RESULTS AND ANALYSIS

A total of 151 women were enrolled between 02 September 2014 and 19 June 2015, 90(59.6%) from Parirenyatwa Hospital and 61(40.4%) from Harare Hospital. All women admitted with puerperal sepsis who met the inclusion criteria (see section 3.4) for the study agreed to participate. Follow up was completed on 01 July 2015.

Socio-demographic data (table 1)

The mean age was 25.1 years with standard deviation of 5.8 years (range 16-44years). Table 1 below shows the social demographic characteristics of the study participants. The majority of women were married (85.4%), had at least 2 children (53.6%), had attained secondary education (76.2%), were unemployed (86.8%), and lived in urban areas (74.8%).

Table 1: Socio-demographic data

Characteristic		**n=151**	**%**
Marital status	Married	129	85.4
	Single	22	14.6
Parity	1	70	46.4
	≥1	81	53.6
Educational status	None	1	0.7
	Primary	24	15.9
	Secondary	115	76.2
	Tertiary	11	7.3
Employment status	Employed	20	13.2
	Unemployed	131	86.8
Residence	Rural	38	25.2
	Urban	113	74.8

Obstetric and Medical Risk Factors (table 2)

Most women (62.9%) had attended ≥4 antenatal care visits in line with the WHO recommended minimum of 4 focused antenatal care visits.

The majority of deliveries were of singleton term fetuses at a hospital. Of the 118 hospital deliveries, 58 had delivered at Parirenyatwa Hospital, 33 at Harare Hospital, 5 at private hospitals and 22 at district hospitals. Parirenyatwa Hospital and Harare Hospitals were the places of delivery for 64% (58/90) and 54% (33/61) of cases of puerperal sepsis they managed respectively. The commonest mode of delivery was Caesarean section (57.6%), mostly as emergency following spontaneous labor of duration <24hours. Only 4 (2.6%) women had assisted vaginal deliveries, all vacuum extractions. Oral misoprostol was the method of induction in all 13 labors that were induced. Few pregnancies had been complicated by pre-labor rupture of membranes (12.6%). Meconium staining of amniotic fluid was noted in 61.6% of pregnancies. Most placentas had been delivered by controlled cord traction or spontaneously (96.0%). Perineal trauma at delivery had occurred in 26.5% of women.

Postpartum hemorrhage was objectively documented to have occurred in 14.6% of women, while 27.2% of women did not report excessive blood loss, but had no documentation. Anemia (hemoglobin <10.0g/dl) was present in just over half of women (51.7%) while hemoglobin status was unknown in 10.6%. Most women were of normal weight (BMI 18.5-24.99kg/m^2) 45.7%, or overweight (BMI 25-29.99kg/m^2) 38.4% (41) . No woman admitted to symptoms or a history of diabetes mellitus or gestational diabetes.

Table 2: Obstetric and medical risk factors

Characteristic		**n=151**	**%**
Booking status	≤3ANC visits	56	37.1
	≥4ANC visits	95	62.9
Number of fetuses	Singleton	147	97.4
	Multiple	4	2.6
Gestational age at delivery	Preterm	20	13.2
	Term	131	86.8
Place of delivery	Hospital	118	78.1
	Local clinic	23	15.2
	Home	10	6.6
Mode of delivery	Normal vaginal	60	39.7
	Assisted vaginal	4	2.6
	Elective caesarean	3	2.0
	Emergency caesarean	84	55.6
Type of labor	Spontaneous	130	86.1
	Induced	13	8.6
	None	8	5.3
Prelabor rupture of membranes	Yes	19	12.6
	No	126	83.4
	Unknown	6	4.0
Labor duration	<24h	81	53.6
	>24h	59	39.1
	None	11	7.3
Meconium staining of amniotic fluid	Yes	93	61.6
	No	50	33.1
	Unknown	8	5.3
Method of placental delivery	Spontaneous	145	96.0
	Manual removal	6	4.0
Perineal trauma	Yes	40	26.5
	No	111	73.5
Postpartum haemorrhage	Yes	22	14.6
	No	88	58.3
	Unknown	41	27.2
Anemia (haemoglobin <10g/dL)	Present	78	51.7
	Absent	57	37.7
	Unknown	16	10.6
Body mass index	Normal (18.5-24.99kg/m²)	69	45.7
	Overweight (25.00-29. 99kg/m²)	58	38.4
	Obese (≥30.00kg/m²)	24	15.9

MICROBIOLOGY

Endocervical swabs isolates (Figure 1 and Appendix 1, table a)

Endocervical swabs were collected for 151 women. Bacteria were isolated from 103 of 151 (68.2%) endocervical swabs, while 48 (31.8%) swabs were negative for growth. One isolate grew on 83(55.0%) swabs, two isolates on 19(12.6%) swabs, while three isolates grew on 1(0.7%) swab. The total number of endocervical swab isolates was 124. Gram negative rods were commonly isolated, most commonly *Escherichia coli* 38(30.6% of total endocervical swab isolates), *Klebsiella pneumoniae* 19(15.3%), 5(4.0%) isolates each of *Pseudomonas aeruginosa* and *Enterobacter species; Citrobacter freundii* 4(3.2%); *Alcaligenes species* 3(2.4%), 2(1.6%) isolates each of *Providencia species, Morganella morganii, Shigella species and Yersinia species; 1(0.8%) isolate each of Salmonella species, Klebsiella oxytoca and Kluyvvera species*. Gram positive cocci isolated were: Coagulase negative staphylococci (CoNS) 9(7.3%), *Staphylococcus aureus* 8(6.5%), *Group D streptococcus* 8(6.5%), *Streptococcus pyogenes* 4(3.2%), *Streptococcus viridans* 2(1.6%) and *Streptococcus agalactae* 1(0.8%). Gram positive rods isolated were: *Corynebacterium species* 5(4.0%) and *Bacillus species* 2(1.6%). No strict anaerobic organisms were isolated.

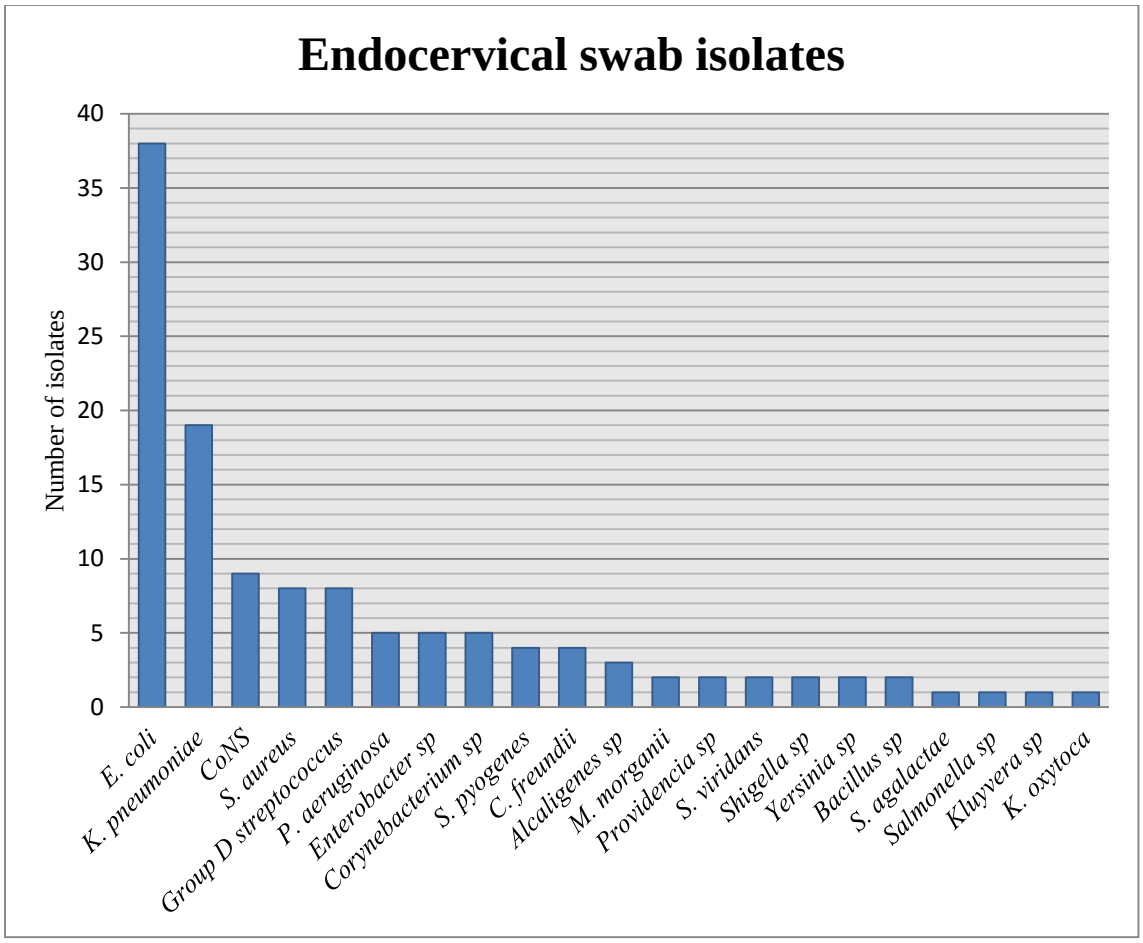

Figure 1

Blood culture isolates (Figure 2 and Appendix 1, table b)

Blood for culture was collected from 150 women. One woman declined further venipuncture after an initial failed attempt. Bacteria were isolated from 14 of 150 bottles and each bottle grew one organism. Overall, blood culture positive rate was 9.3%. This decreased to 3.3% after excluding *Bacillus sp,* CoNS and fungus as possible contaminants. Isolates obtained were *Bacillus sp* 5(35.7% of blood culture isolates), CoNS 3(21.4%), *E. coli* 2(14.3%), *S. aureus* 1(7.1%), *Alcaligenes sp* 1(7.1%), *Moraxella sp* 1(7.1%) and fungus 1(7.1%). Both *E. coli grew* on day 1. *Moraxella sp* and *S. aureus* grew on day 5, while A*lcaligenes sp* was noted on day 10. Only one

woman had the same organism, *E. coli*, isolated from both the blood culture and endocervical swab, with similar antibiotic susceptibility profile.

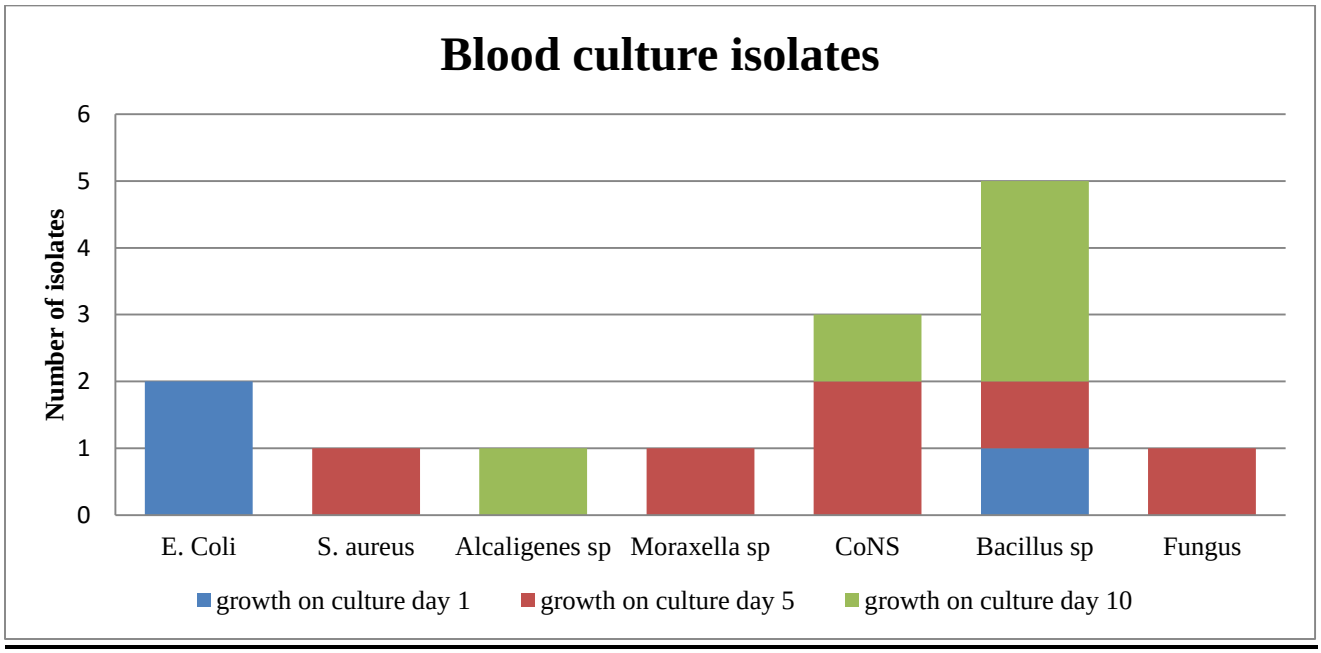

Figure 2

Antibiotic use prior to specimen collection

Specimen collection before administration of antibiotics was achieved in only 23/151(15.2%) women as the other women had already received antibiotics at the primary care center or during screening in casualty or as prophylaxis prior to and post caesarian section. Ceftriaxone and metronidazole had been administered in 74.8% and 78.1% respectively. Other antibiotics given were benzyl penicillin(7.3%), ampicillin(1.3%), cloxacillin(0.7%), amoxicillin(4.0%), chloramphenicol(5.6%), gentamycin(2.6%), ciprofloxacin(0.7%), clindamycin(0.7%), erythromycin(0.7%).

Antibiotic Susceptibility Profiles of Endocervical swab isolates (Figures 3-9 and Appendix 1, table c)

High level resistance to the 7 empiric antibiotics tested was noted for most gram negative isolates. Levels of antibiotic resistance for *E. coli*, the most prevalent isolate were: ceftriaxone (60.5%), penicillin (94.7%), gentamycin (50%), clindamycin (92.1%), erythromycin (86.8%), chloramphenicol (39.5%) and ciprofloxacin (34.2%). Antibiotic resistance levels for *K. pneumonia*, the second most prevalent isolate, were: ceftriaxone (68.4%), penicillin (89.5%), chloramphenicol (63.2%), gentamycin (63.2%), clindamycin (100%), ciprofloxacin (42.1%), and erythromycin (94.7%). *Enterobacter sp* resistance was 100% to gentamycin, clindamycin and erythromycin; 80% to ceftriaxone and penicillin; and 40% to chloramphenicol and ciprofloxacin.

Gram positive organisms were generally susceptible to the empiric antibiotics. *S. pyogenes* isolates were fully susceptible to penicillin, chloramphenicol, clindamycin and erythromycin; while resistance was 50% to ceftriaxone and 25% to gentamycin and ciprofloxacin. *S. aureus* isolates exhibited 50% resistance to ceftriaxone, gentamycin, clindamycin and erythromycin; 37.5% resistance to penicillin and 25% resistance to chloramphenicol and ciprofloxacin. CoNS were fully susceptible to clindamycin; while resistance was 55.6% to penicillin, 44.4% to ceftriaxone, chloramphenicol and erythromycin, and 33.3% to gentamycin and ciprofloxacin. *S. agalactae* was susceptible to all the antibiotics except gentamycin. *S. viridans* isolates were fully susceptible to ceftriaxone, clindamycin and erythromycin, while resistance to chloramphenicol was 50%.

Blank spaces in graphs mean the organism was not tested for susceptibility to the antibiotic. Antimicrobial susceptibility of *Bacillus sp* was not done, see section 3.7.3.4.d.

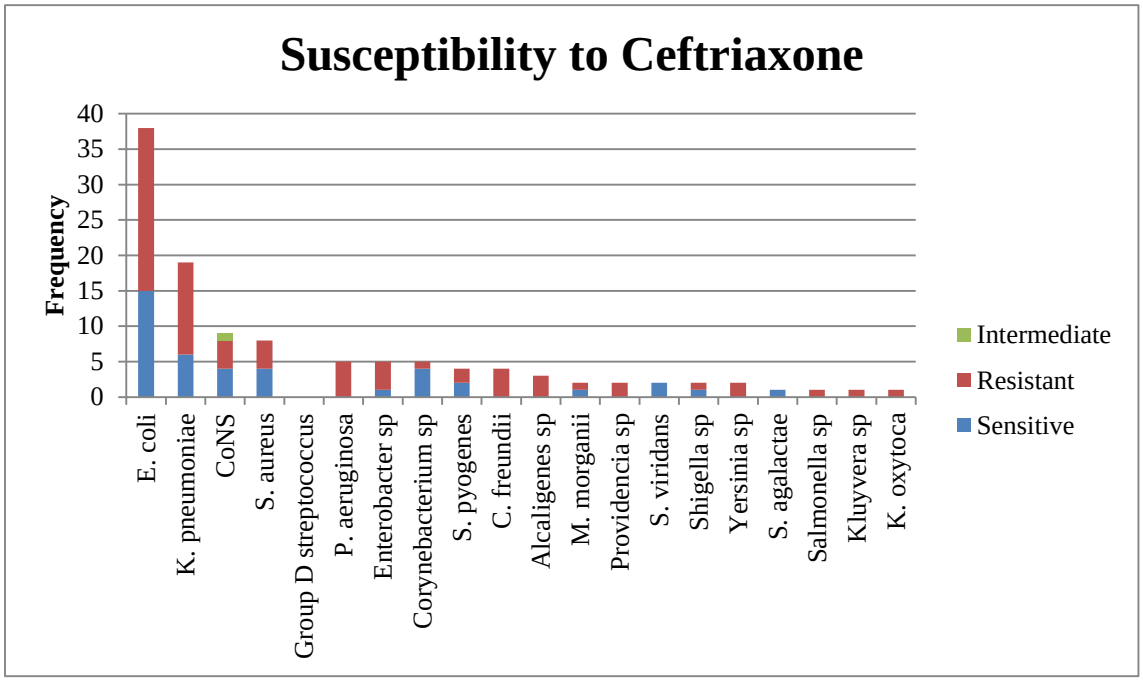
Susceptibility to Ceftriaxone
Frequency
40
35
30
25
20
15
10
5
0
E. coli
K. pneumoniae
CoNS
S. aureus
Group D streptococcus
P. aeruginosa
Enterobacter sp
Corynebacterium sp
S. pyogenes
C. freundii
Alcaligenes sp
M. morganii
Providencia sp
S. viridans
Shigella sp
Yersinia sp
S. agalactae
Salmonella sp
Kluyvera sp
K. oxytoca
Intermediate
Resistant
Sensitive

Figure 3

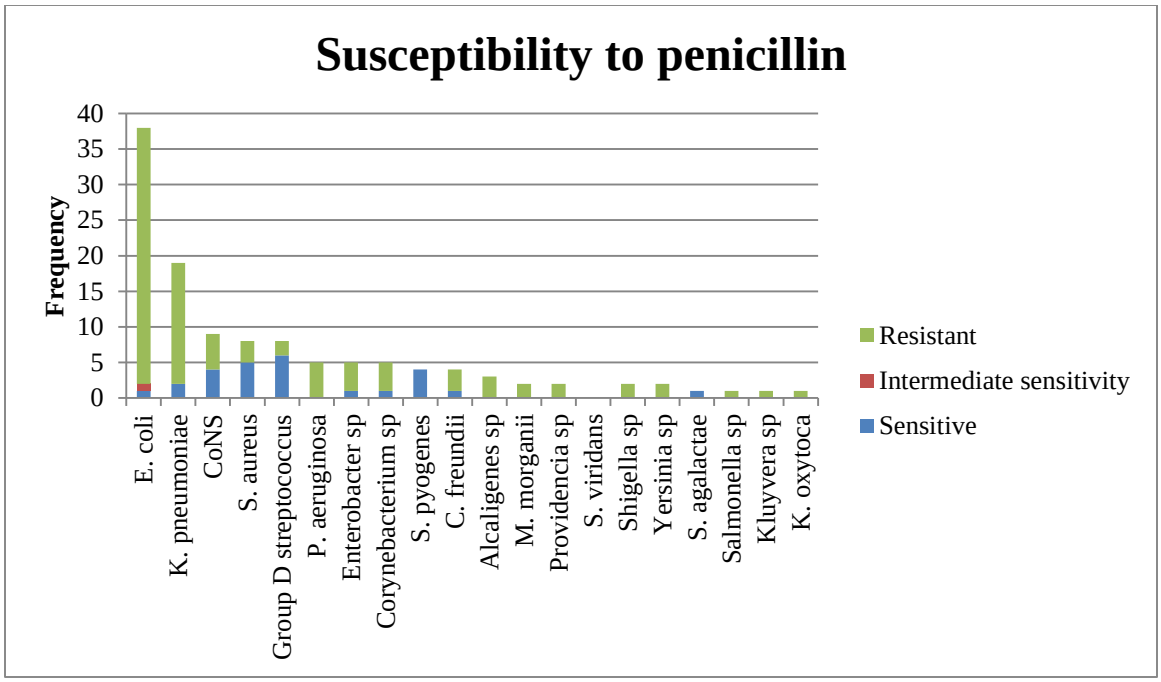

Figure 4

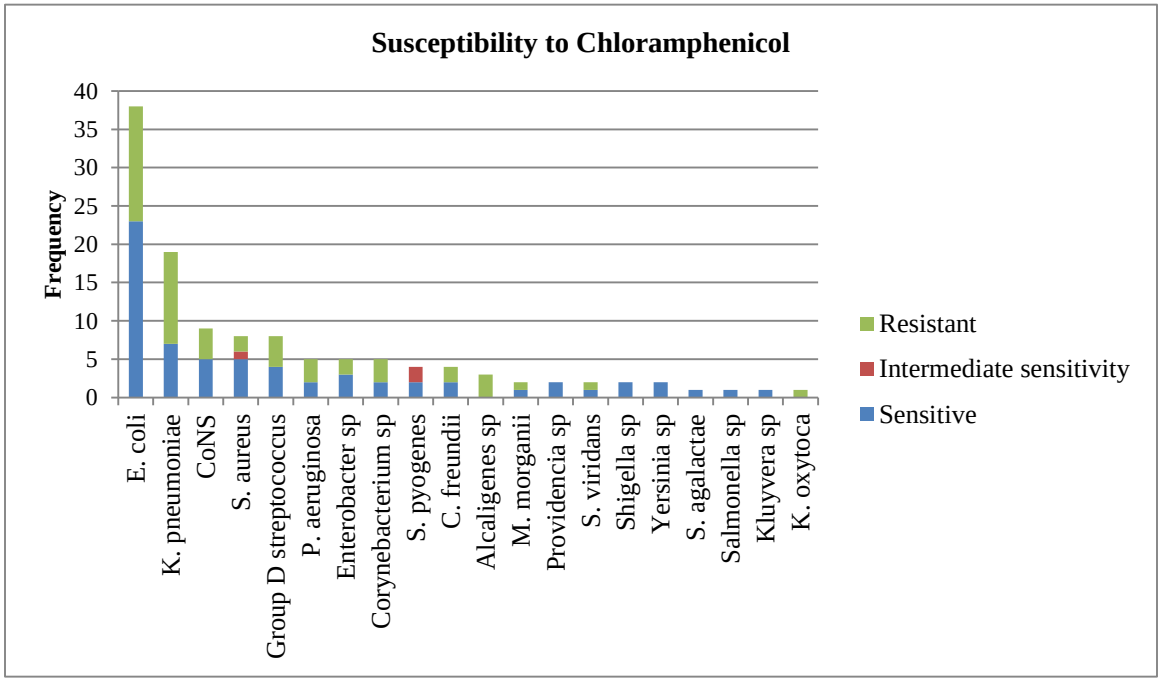

Figure 5

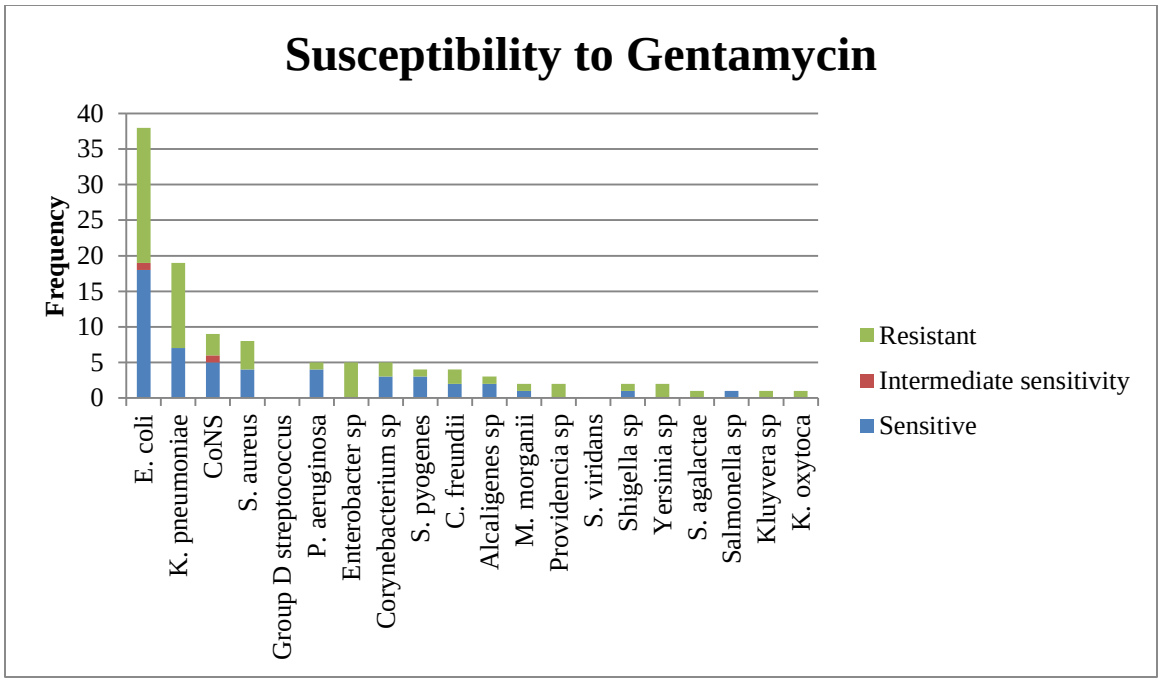

Figure 6

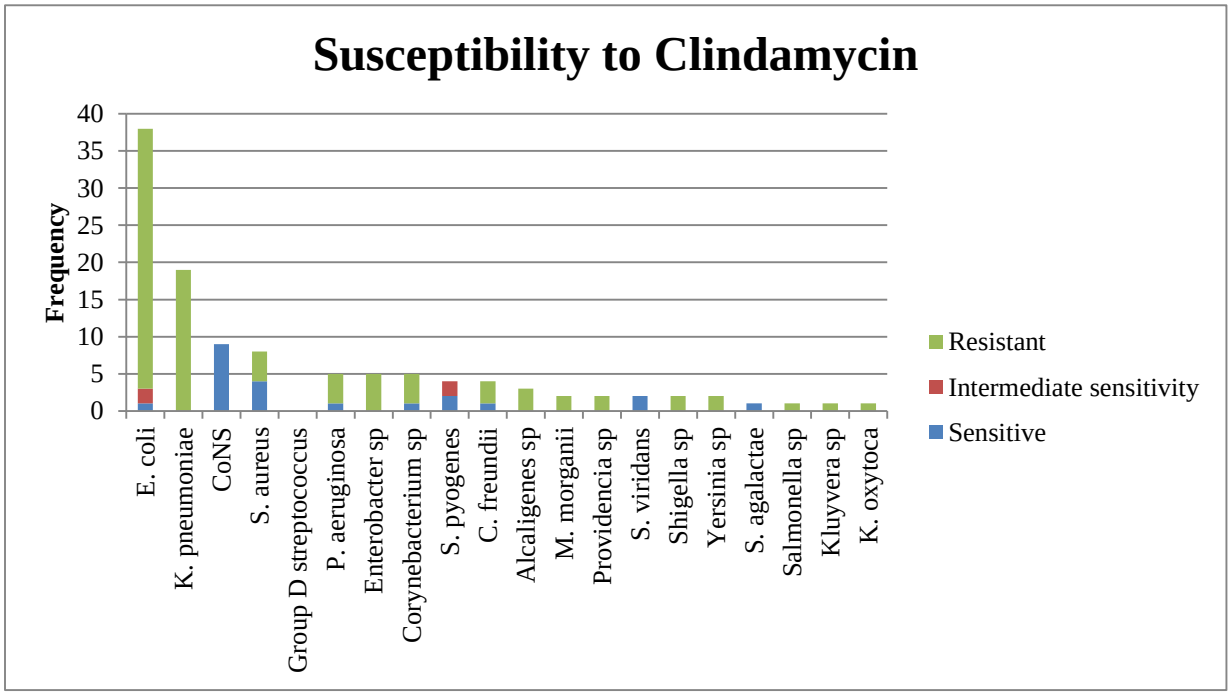

Figure 7

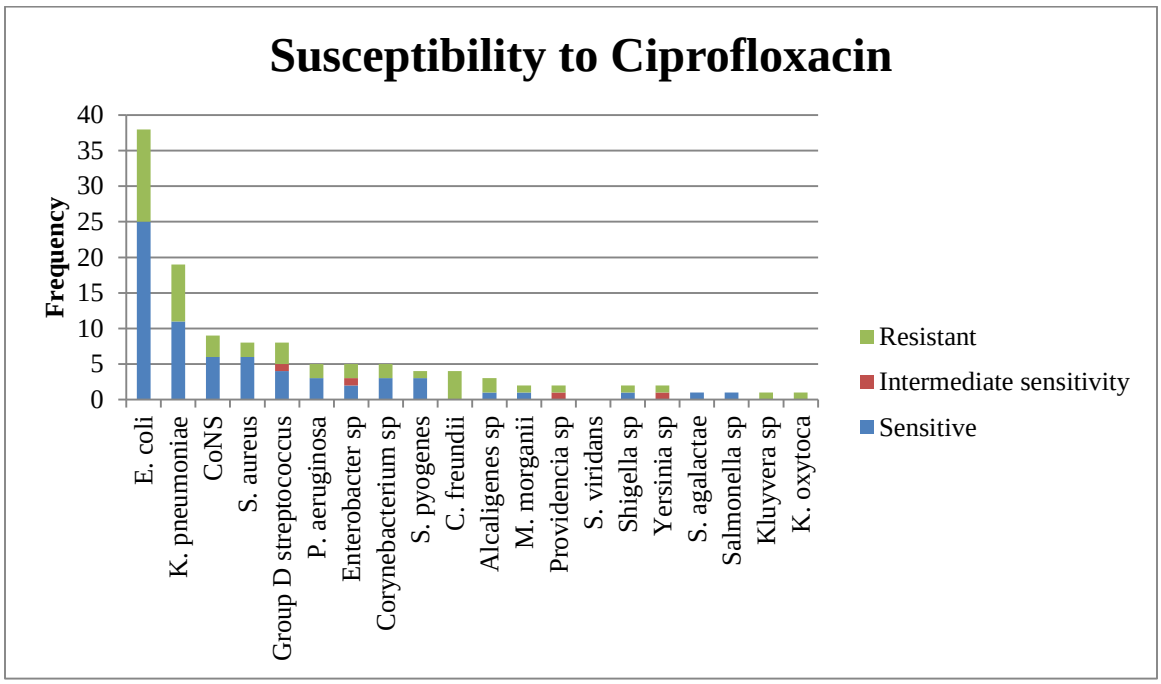

Figure 8

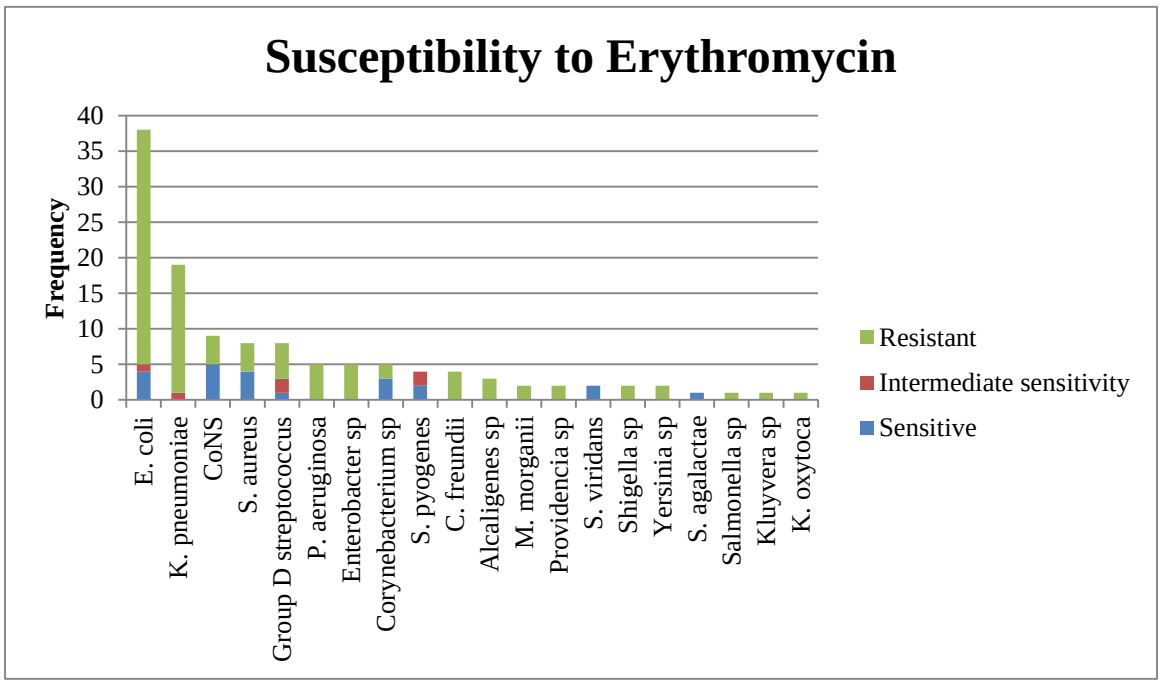

Figure 9

Blood culture antibiotic susceptibility profiles (table 3)

E. coli isolates were fully susceptible to ceftriaxone and ciprofloxacin, while resistance was 50% to clindamycin and erythromycin; and 100% to gentamycin, chloramphenicol and penicillin G.

S. aureus was resistant to all 7 antibiotics. *Alcaligenes sp* was susceptible to ceftriaxone, chloramphenicol, gentamycin and ciprofloxacin.

Table 3: Antibiotic susceptibility of blood culture isolates

		CRO		P		C		Gn		Clin		CIP		E	
Isolate	Susceptibility	No	%	No	%	No	%	No	%	No	%	No	%	No	%
E. Coli	S	2	100	0	0	0	0	0	0	1	50	2	100	1	50
	R	0	0	2	100	2	100	2	100	1	50	0	0	1	50
S. aureus	S	0		0		0		0		0		0		0	
	R	1		1		1		1		1		1		1	
Alcaligenes sp	S	1		0		1		1		0		1		0	
	R	0		1		0		0		1		0		1	

Key:
CRO- ceftriaxone, P- penicillin, C- chloramphenicol, Gn- gentamycin, Clin- clindamycin, CIP- ciprofloxacin, E- erythromycin, No- number
S- Susceptible
I- Intermediate susceptibility
R- Resistant
Susceptibility not tested for *Bacillus sp,* CoNS, Moraxella sp, and fungus. See section 3.7.3.4.d.

Multi-drug resistant organisms (Figure 10 and appendix 1, table d)

Organisms with acquired non-susceptibility to at least one agent in three or more antimicrobial categories above were designated as multidrug resistant organisms (MDRO) (32). MDROs were tested for susceptibility to carbapenems (imipenem and meropenem). There were fifteen MDROs. All 14 MDRO isolated from endocervical swabs were gram negative bacilli, most commonly *K. pneumoniae*. The one MDRO isolated from the blood stream was *S. aureus.* MDRO were common,

representing 32% of *K. pneumoniae*, 5% of *E. coli*, 50% of *M. morganii*, 20% of *Enterobacter sp*, 20% of *P.aeruginosa*, 33% of *Alcaligenes sp*, 25% of *C. freundii* and 11% of *S. aureus.*

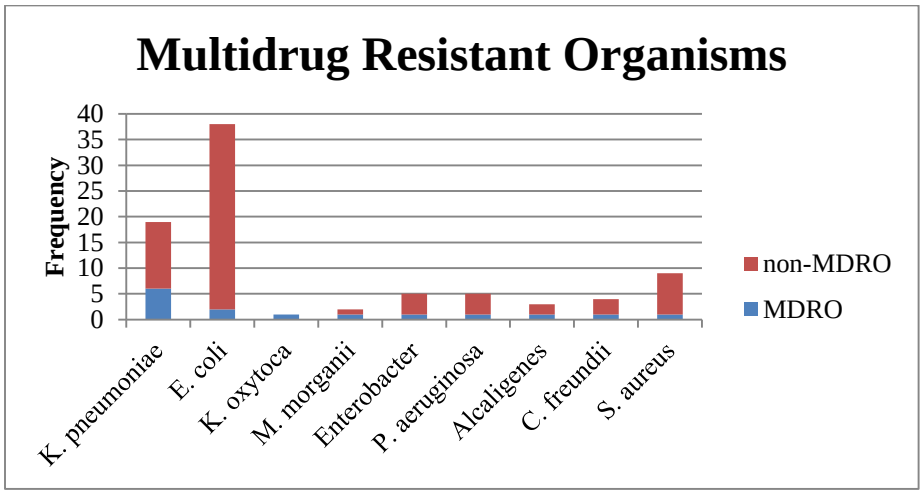

Figure 10

Of the six MDRO *K. pneumoniae*, 3 isolates were susceptible to both meropenem and imipenem, 2 isolates were resistant to meropenem but susceptible to imipenem, while 1 isolate was resistant to both carbapenems. Both *E. coli* were susceptible to meropenem. Imipenem susceptibility was confirmed for 1 isolate, but not tested for the other, see section **3.7.3.4.d** . *S. aureus* was resistant to meropenem but susceptible to imipenem. *M. Morganii, Alcaligenes sp, P. aeruginosa, C. freundii*, were susceptible to both carbapenems. Imipenem susceptibility was not tested for *K. xytoca* and *Enterobacter sp*, see section **3.7.3.4.d**.

HIV Status

Valid HIV status results were obtained for all 151 women. Thirty-three women (21.9%) were HIV positive (Figure 11). Valid CD4 counts were available for 29 of 33 women. The majority of women, 15 (45.5%), had mild (CD4 350-499/mm³) to advanced immunosuppression (CD4 200-349/mm³). There were 7(21.2%) women in each of the insignificant (CD4 > 500/mm³) and severe immunosuppression (CD4 <200/mm³) categories.

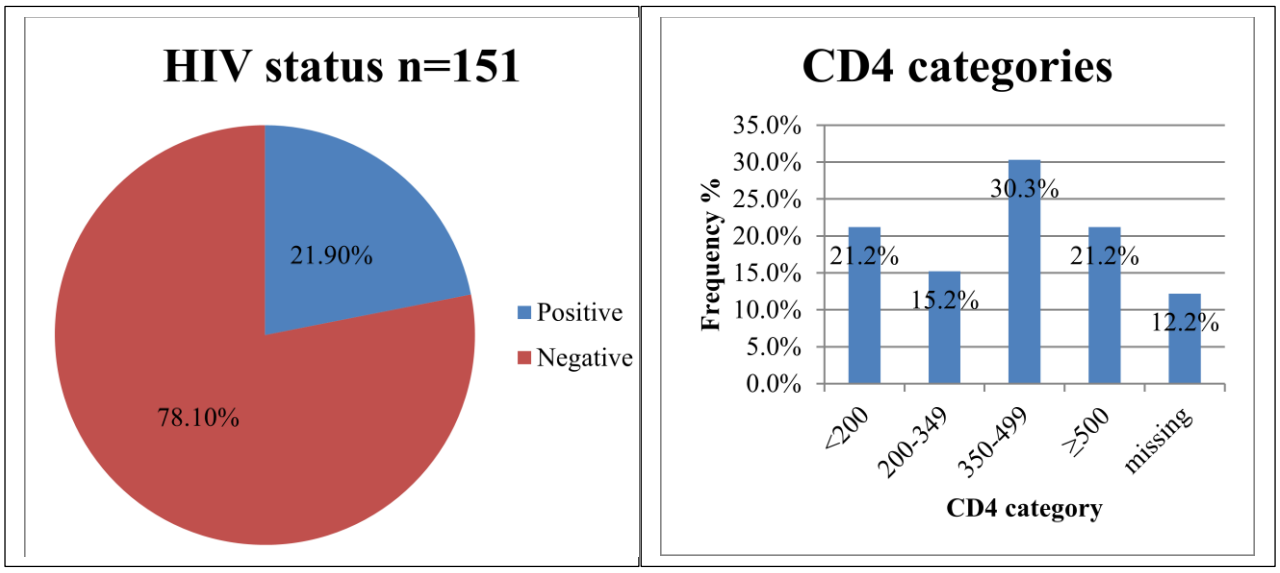

Figure 11: HIV status and CD4 Count

Twenty-three (69.7%) women were on treatment with antiretroviral therapy (ART). HIV was diagnosed for the first time at admission for puerperal sepsis in the 10 (30.3%) women who were not on ART. Eleven women had been on ART for >6months, while 12 had been on ART for <6 months

Association of HIV status with microbiology of puerperal sepsis (table 4 and table5)

Enterobacter sp were more prevalent in the genital tract of HIV-infected than HIV-uninfected women 9.1% vs 1.7% (p=0.04). There was no statistically significant difference between the two groups with regard to the other organisms isolated from the blood stream and the endocervix. There was no difference between number of isolates per swab with 1 isolate growing in 86.4% vs 87.9% (p= 0.55); 2 isolates in 12.7% vs 12.1% (0.93) ; 3 isolates in 0.8% vs 0% (p=0.596) of HIV-uninfected and HIV-infected women respectively. The proportion of MDRO was no different between the 2 groups, 9.1% compared to 11.9% (p=0.78) of HIV-infected women and of HIV-uninfected respectively.

Table 4: Association of blood culture isolates with HIV status.

	HIV status				**p-value**
	Positive(n=32)		**Negative(n=118)**		
Blood culture isolate	Number	%	Number	%	
E. coli	0	0	2	1.7	0.46
Bacillus sp	1	3.0	4	3.4	0.94
S. aureus	0	0	1	0.8	0.60
Moraxella sp	0	0	1	0.8	0.60
Alcaligenes sp	0	0	1	0.8	0.60
CoNS	2	6.3	1	0.8	0.05
Fungal contamination	0	0	1	0.8	0.60
No growth	29	90.6	107	90.7	1.00

Table 5: Association of endocervical swab isolates with HIV status.

Endocervical swab isolate	HIV status				p-value
	Positive(n=33)		Negative(n=118)		
	Number	%	Number	%	
E. coli	11	33.3	27	22.3	0.22
K. pneumonia	6	18.2	13	11.0	0.27
K. oxytoca	0	0	1	0.8	0.60
M. morganii	0	0	2	1.7	0.45
Providencia sp	1	3.0	1	0.8	0.33
P. aeruginosa	0	0	5	4.2	0.23
Alcaligenes sp	0	0	3	2.5	0.35
Bacillus sp	0	0	2	1.7	0.45
C. freundii	1	3	3	2.5	0.88
S. aureus	1	3	7	5.9	0.51
CoNS	1	3	8	6.8	0.42
S. pyogenes	1	3	3	2.5	0.88
Corynebacterium sp	0	0	5	4.2	0.76
Enterobacter sp	3	9.1	2	1.7	0.04
Salmonella sp	0	0	1	0.8	0.60
Kluyvera sp	1	3	0	0	0.06
Yersinia sp	0	0	2	1.7	0.45
Group D streptococcus	2	6.1	6	5.1	0.83
S. viridans	0	0	2	1.7	0.45
S. agalactae	0	0	1	0.8	0.60
Shigella sp	0	0	2	1.7	0.45
No growth	9	27.3	39	33.1	0.53

Presentation of puerperal sepsis and clinical outcomes

Median time from delivery to onset of puerperal sepsis was 6 days, IQR 3-11days (range 0-42days). The commonest presenting symptoms were abnormal vaginal discharge and pelvic pain, see Figure 12.

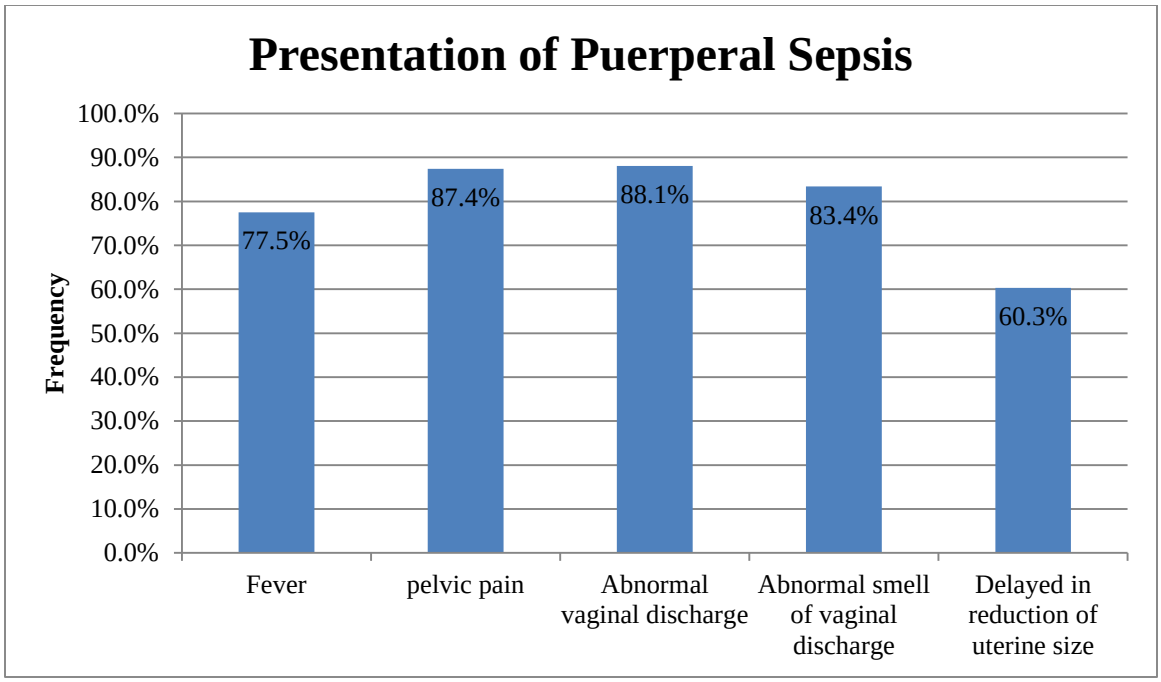

Figure 12

Eighty-one women (53.6%) experienced at least one complication. Two or more complications occurred in 13.9% of women. Surgical wound dehiscence was most prevalent occurring in 39.1% of women, see Figure 13. Eleven women died, a case fatality rate of 7.3%.

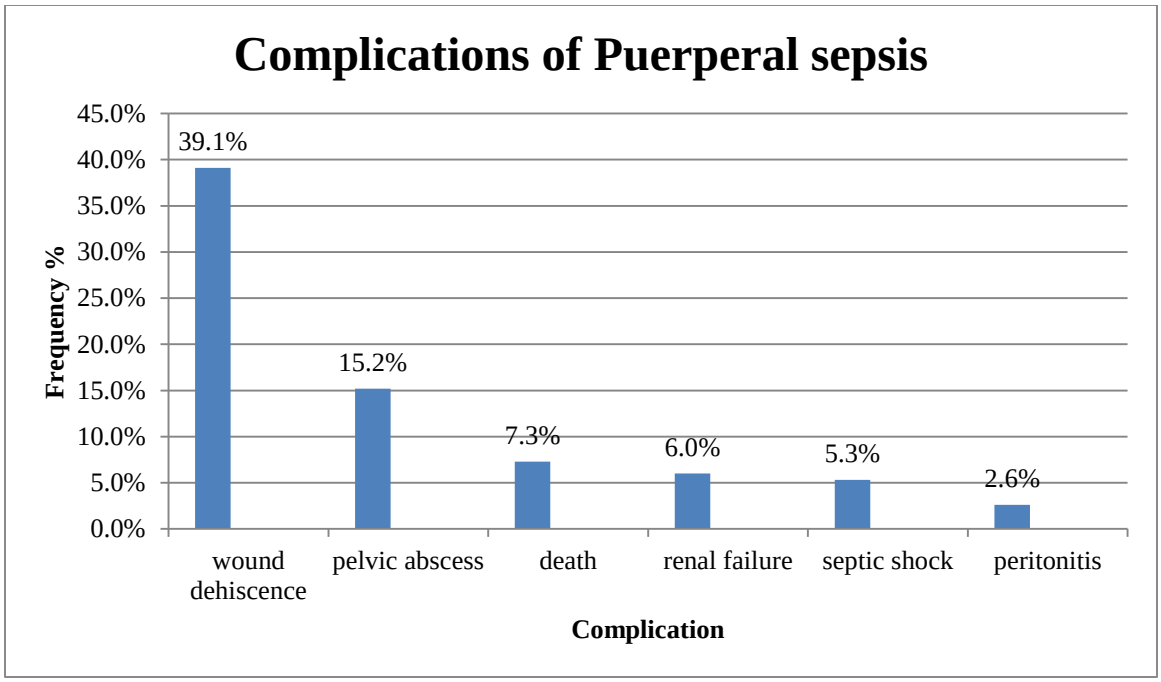

Figure 13

Laparotomy was performed in 25 (16.6%) women. A pelvic abscess was confirmed by the presence of pus in the pelvis in 23 women. There was no obvious pus in the pelvis in two women; one had a grossly necrotic uterus and anterior abdominal wall tissue planes, while the other had peritonitis. Laparotomy was performed twice in four women (2 for re-accumulation of pus, 2 for colostomy complications), while the fifth woman died whilst awaiting re-laparotomy for burst abdomen. Hysterectomy for gross pelvic sepsis was performed in 1 woman.

Admission into the intensive care unit (ICU) was required for eighteen women (11.9%). The median length of ICU stay was 3 days, IQR 2.75-5.25 days (range 1 to 12days)). Median length of hospital stay was 8days, IQR 4-16 days (range1-51days).

Association of antibiotic susceptibility with clinical outcomes (table 6 and Figure 14)

MDRO were found in 12 women, (one woman had 3 MDRO, and another had 2 MDRO, while ten women had one each). Mean length of hospital stay was significantly longer in women with MDRO compared to those without MDRO, 23.0 days vs 10.5 days respectively (p=0.009). There was a trend towards higher complication rates (pelvic abscess, septic shock, wound dehiscence, death), need for laparotomy and ICU admission in women with MDRO than those without MDRO, but this did not reach statistical significance.

Table 6: Association of presence of MDRO with clinical outcomes of puerperal sepsis

	MDRO (n=12)		**Non-MDRO (n=139)**		**p-value**
	Number	%	number	%	
Pelvic abscess	4	33.3	19	13.7	0.07
Septic shock	1	8.3	7	5.0	0.42
Wound dehiscence	5	41.7	54	38.8	0.85
Renal failure	0	0	9	6.5	0.36
Peritonitis	0	0	4	2.9	0.55
Death	2	16.7	9	6.5	0.19
Laparotomy	4	33.3	21	15.1	0.10
ICU admission	3	25.0	15	10.8	0.14
Length of hospital stay (mean)		23.0		10.5	0.009

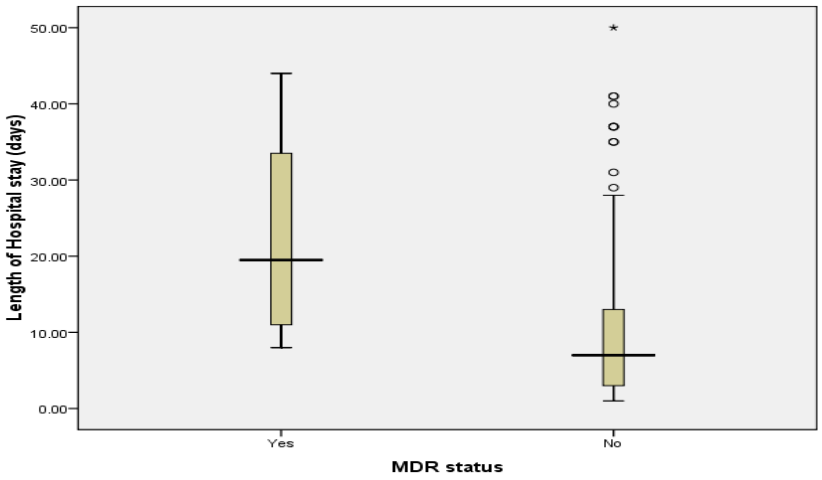

Figure 14: Association of presence of MDRO and length of hospital stay

Association of HIV status with presentation and clinical course of puerperal sepsis (table 7)

All clinical outcomes were not significantly different between HIV infected and HIV uninfected women.

Table 7: Association of HIV status with clinical presentation of puerperal sepsis

	HIV positive (n=33)		**HIV negative (n=118)**		**p-value**
	Number	%	Number	%	
Pelvic abscess	4	12.1	19	16.1	0.14
Septic shock	2	6.1	6	5.1	0.83
Wound dehiscence	11	33.3	48	40.7	0.45
Renal failure	4	12.1	5	4.2	0.09
Peritonitis	1	3.0	3	2.5	0.88
Death	4	12.1	7	5.9	0.22
Laparotomy	5	15.2	20	16.9	0.80
ICU admission	6	18.2	12	10.2	0.21
Mean time to onset of puerperal sepsis(days)		9.3		8.3	0.10
Mean length of hospital stay(days)		11.2		11.6	0.43

Association of immunological status (CD4 cell count) with clinical presentation of puerperal sepsis (tables 8 and 9)

The frequency of fever, pelvic pain, abnormal vaginal discharge, foul-smelling vaginal discharge, and delay in rate of reduction of size of the uterus was no different between the immunological groups. Severe immunosuppression (CD4 <200/mm^3, n=7) resulted in greater need for laparotomy, 42.9% vs 4.5% (p=0.01), and prolonged mean hospital stay, 19.0days vs 10.2days (p=0.03), compared to mild-advanced or insignificant immunosuppression (CD4 >200/mm^3, n=22). There was a trend towards earlier onset of sepsis; and higher rates of pelvic abscess, septic shock, wound dehiscence, peritonitis, death and need for ICU admission in women with severe immunosuppression though this did not reach statistical significance. There were no significant differences in clinical presentation and clinical course between women with mild-advanced and those with insignificant immunosuppression.

Table 8: Association of immunological status with presentation of puerperal sepsis

Clinical Feature	CD4 count				P-value
	<200 (n=7)		≥200 (n=22)		
	Number	%	Number	%	
Fever	7	100.0	18	81.8	0.22
Pelvic pain	7	100.0	18	81.8	0.22
Abnormal vaginal discharge	5	71.4	19	86.4	0.36
Abnormal smell of vaginal discharge	5	71.4	18	81.8	0.36
Delay in reduction of uterine size	3	42.9	12	54.5	0.59
Pelvic abscess	2	28.6	2	9.1	0.19
Septic shock	1	14.3	1	4.5	0.37
Wound dehiscence	4	57.1	7	31.8	0.23
Renal failure	0	0.0	4	18.2	0.22
Peritonitis	1	14.3	0	0.0	0.07
Death	2	28.6	2	9.1	0.19
Laparotomy	3	42.9	1	4.5	0.01
ICU admission	3	42.9	3	13.6	0.10
Mean time to onset of sepsis(days)	2.5		11.8		0.07
Mean length of hospital stay(days)	19.0		10.2		0.03

Table 9: Association of immunological status with presentation of puerperal sepsis

Clinical feature	CD4 count				P-value
	200-500 (n=15)		>500 (n=7)		
	Number	%	Number	%	
Fever	12	80.0	6	85.7	0.75
Pelvic pain	13	86.7	5	71.4	0.39
Abnormal vaginal discharge	14	93.3	5	71.4	0.16
Abnormal smell of vaginal discharge	8	53.3	4	57.1	0.87
Delay in reduction of uterine size	8	53.3	4	57.1	0.87
Pelvic abscess	2	13.3	0	0.0	0.31
Septic shock	1	6.7	0	0.0	0.48
Wound dehiscence	4	26.7	3	42.9	0.45
Renal failure	3	20.0	1	14.3	0.75
Peritonitis	0	0.0	0	0.0	-
Death	1	14.3	1	14.3	-
Laparotomy	1	6.7	0	0.0	0.31
ICU admission	2	13.3	1	14.3	0.95
Mean time to onset of sepsis (days)		13.5		6.5	0.15
Mean length of hospital stay (days)		11.6		7.1	0.30

Association of use of antiretroviral therapy with presentation of puerperal sepsis (table 10 and 11)

Use of antiretroviral therapy and duration of use were not related to the presentation and clinical course of puerperal sepsis.

Table 10: Association of use of antiretroviral therapy with presentation of puerperal sepsis

Clinical feature	Use of ART				P-value
	Yes (n=23)		No (n=10)		
	Number	%	Number	%	
Fever	19	82.6	9	90.0	0.59
Pelvic pain	19	82.6	9	90.0	0.59
Abnormal vaginal discharge	19	82.6	9	90.0	0.59
Abnormal smell of vaginal discharge	18	78.3	9	90.0	0.42
Delay in rate of reduction of uterine size	11	47.8	6	60.0	0.52
Pelvic abscess	3	13.0	1	10.0	0.81
Septic shock	1	4.3	1	10.0	0.53
Wound dehiscence	8	34.8	3	30.0	0.79
Renal failure	3	13.0	1	10.0	0.81
Peritonitis	1	4.3	0	0.0	0.50
Death	3	13.0	1	10.0	0.81
Laparotomy	4	17.4	1	10.0	0.59
ICU admission	5	21.7	1	10.0	0.64
Mean Time to onset of sepsis (days)		10.2		7.8	0.72
Mean length of hospital stay (days)		9.8		14.5	0.32

Table 11: Association of duration of use of antiretroviral therapy with presentation of puerperal sepsis

Clinical feature	Duration of use of ART				P-value
	<6 months (n=12)		> 6 months (n=11)		
	Number	%	Number	%	
Fever	9	75.0	10	90.9	0.32
Pelvic pain	11	91.7	8	72.7	0.23
Abnormal vaginal discharge	11	91.7	8	72.7	0.23
Abnormal smell of vaginal discharge	9	75.0	9	81.8	0.69
Delay in rate of reduction of uterine size	7	16.7	1	9.1	0.59
Pelvic abscess	2	16.7	1	9.1	0.59
Septic shock	0	0.0	1	9.1	0.48
Wound dehiscence	4	33.3	4	36.4	0.88
Renal failure	1	8.3	2	18.2	0.59
Peritonitis	1	8.3	0	0.0	0.33
Death	1	8.3	2	18.2	0.59
Laparotomy	3	25.0	1	9.1	0.59
ICU admission	3	25.0	2	18.2	0.69
Mean time to onset of sepsis (days)		12.1		7.9	0.33
Mean length of hospital stay (days)		11.7		7.7	0.40

Risk factors for bacteremia

Manual removal of placenta was associated with a non-significant trend towards a higher risk for bacteremia [RR 4.893, p=0.02 (CI= 0.98-24.38)] (Figure 15). Other demographic, obstetric and medical factors were not associated with development of bacteremia.

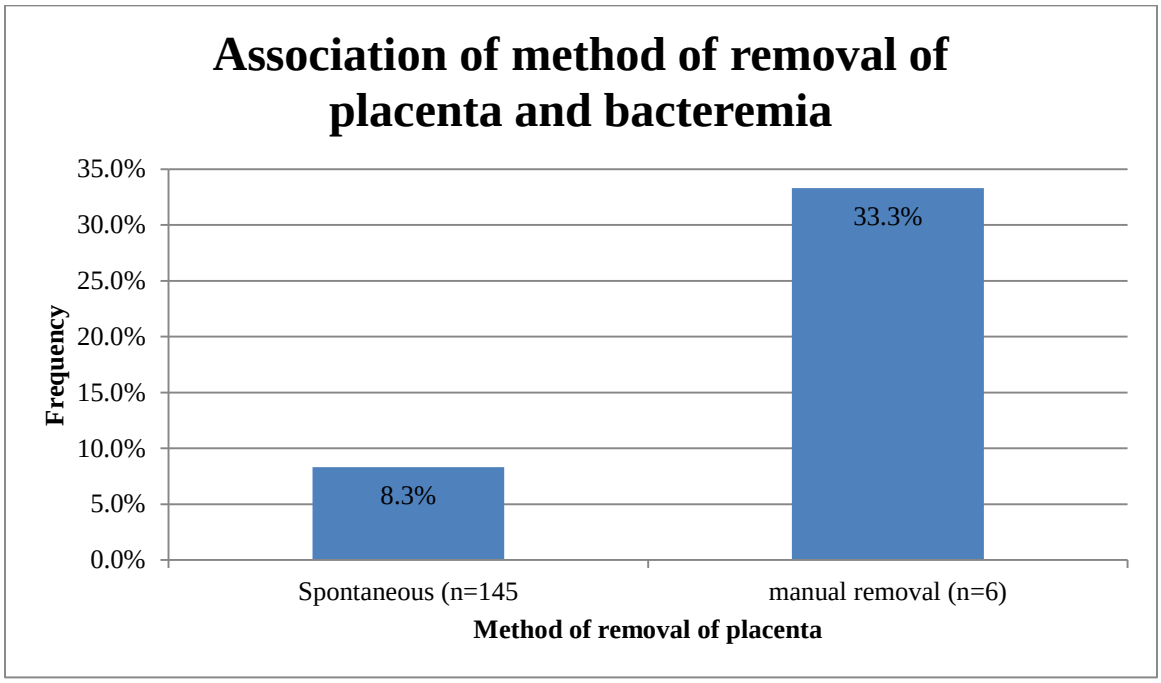

Figure 15

CHAPTER 5

5.1 DISCUSSION

The majority of women managed for puerperal sepsis at Parirenyatwa and Harare Hospitals had delivered at a hospital (78.1%), by caesarean section (57.6%), raising concerns about nosocomial infections. Most cases of postpartum endometritis follow operative delivery. In a Cochrane review, the rate of puerperal infections was 2.5% in women who had a vaginal delivery while the average rate of endometritis was 9.2% in those women undergoing elective caesarean section and 28% in women undergoing non-elective caesarean section(51).

Bacteria were isolated in 68.2% of endocervical swabs, a rate consistent with other studies in which laboratory-confirmed infection was reported for 63.8% of cases of severe maternal sepsis in a United Kingdom case control study (52), and in up to 80% of cases in other studies from resource limited settings (7,14,16,17). Bacteria were isolated in 9.3% of blood culture specimens, but significant isolates were obtained in 3.3% of specimens. *Bacillus sp* and *Coagulase negative staphylococci* are part of the indigenous flora of the skin, and their isolation in a blood culture may simply represent contamination. However, both may represent significant growth in special circumstances such as immunosuppression, intravenous drug abuse, indwelling foreign devices (e.g. - intravascular catheters, pacemakers, central nervous system shunts) and sickle cell disease which should be confirmed by sustained growth on repeat blood cultures. Although 12-25% of CoNS and 5-10% of *Bacillus sp* isolated from blood cultures are due to significant blood stream infections, CoNS and *Bacillus sp* were considered contaminants because repeat cultures to confirm infection could not be done(48–50,53).

Blood culture positive rate was not significantly different from findings of a review by Williams and Pastorek in which rate of bacteremia was 5-20% in studies investigating postpartum endomyometritis(8). Failure to identify a causative organism is expected. Manual removal of the placenta was the single risk factor that was associated with development of bacteremia, though it did not reach statistical significance. This may be associated with introduction of nosocomial pathogens to the raw placental surface in the uterus via infected hands.

Gram negative enterobacteriacae were commonly isolated from the endocervix, *E. coli* and *K. pneumoniae* representing 45.9% of the total isolates. *E. coli* were also the commonest significant cause of bacteremia, being isolated in 2 specimens in this study. *E. coli* has been reported as the commonest cause of severe maternal sepsis originating from the genital tract (15,52). Furthermore, genes associated with high virulence such as *hly-, cnf-, pap-* and *iro*N-genes have been found significantly more frequently in *E. coli* strains isolated from vaginal and/or endocervical samples of pregnant women. These predispose to severe maternal sepsis(54). Group A streptococci, an important cause of severe maternal sepsis leading to septic shock and death in the developed world(52), were identified in only 3.2% of genital specimens in this study implying that it may not be a significant pathogen in our setting or it is highly susceptible to empiric antimicrobials.

High level resistance by most gram negative isolates form the endocervix to the first line regimens for the treatment of puerperal sepsis was demonstrated in this study. There is emerging resistance by both gram negative and gram positive bacteria to multiple antimicrobial agents including to meropenem and imipenem, the most readily available carbapenems used for salvage therapy for resistant infections in both public and private hospitals in Zimbabwe. These multidrug resistant

organisms (MDRO), defined as organisms with acquired non-susceptibility to at least one agent in three or more antimicrobial classes (32), are a significant threat to public health. Furthermore, extremely drug-resistant enterobacteriacae are increasingly isolated worldwide (55,56) placing additional strain on limited resources.

Puerperal sepsis is associated with high rates of maternal morbidity and mortality. Laparotomy was needed in 16.6% of women with puerperal sepsis, of which 20% of them had a repeat laparotomy. Case fatality rate in this prospective cohort of hospitalized women was 7.3%. A review of the global burden of maternal sepsis in the year 2000 reported case fatality rates of 4-50% in Sub-Saharan African countries, the wide differences being probably due to different definitions of puerperal sepsis and whether the study was hospital based or population based (1).

MDRO significantly increased length of hospital stay, and there was a clinically important trend towards higher rates of pelvic abscess, septic shock, death, need for laparotomy and ICU admission. Several investigators have noted increased length and cost of hospital stay and mortality associated with infections due to multidrug-resistant *S. aureus* and gram-negative bacilli such as *E. coli*, *K. pneumoniae*, *Enterobacter sp*, *P. aeruginosa*, and *Acinetobacter sp* (57–62).

The prevalence of HIV infection was higher in this cohort of women with puerperal sepsis (21.9%) than in the general Zimbabwean population (15%) (63). HIV infection predisposes to the development of puerperal sepsis. In a systematic review, Calvert found that HIV-infection was associated with a three-fold increased risk of a puerperal sepsis in women after a vaginal or caesarian delivery; a figure that increased to nearly six amongst studies only including women who delivered by caesarian section(9).

A significant number of women are still going through pregnancy (30.3%) without HIV counseling and testing, placing themselves at risk of HIV/AIDS related illness and their babies at risk of maternal to child transmission of HIV. A trend towards universal access to ART for HIV infected pregnant women was confirmed in this cohort where all women who were known to be HIV infected had commenced antiretroviral therapy.

Enterobacter sp were more prevalent in HIV-infected women (p=0.04). In a retrospective review by Manifredi, *Enterobacter sp* were more frequently isolated in severely immunosuppressed HIV infected patients, demonstrating a possible increased pathogenicity of *Enterobacter sp* in HIV infection (64). However, HIV infection did not influence colonization with the rest of the organisms, nor did it increase the rate of polymicrobial colonization of the genital tract or blood stream as well as the proportion of MDRO in this study. The microbiology of organisms causing upper genital tract infections has been found to be similar among HIV infected and uninfected women by other investigators (25,65). In one case-control study, HIV infected women exhibited more severe clinical manifestations of pelvic inflammatory disease with no effect on microbial cause or response to therapy (25).

Overall, being infected with HIV did not appear to significantly alter clinical course of puerperal sepsis. Since only a minority of HIV-positive women with puerperal sepsis in this study had severe immunosuppression of CD4 count <200/mm³ (21.2%), the impact of HIV on clinical disease was not apparent. After sub-group analysis, HIV- associated severe immunosuppression was evidently a risk factor for prolonged hospital stay and need for laparotomy. A clinically significant trend towards earlier onset of puerperal sepsis and higher complication rates was observed with severe immunosuppression. This is consistent with findings by Cohen who showed that a CD4 <14% was associated with an increased likelihood of a tubo-ovarian abscess and a longer hospital stay in women with acute salpingitis (65). Use of antiretroviral therapy did not independently affect clinical manifestations of puerperal sepsis in this cohort.

5.2 Study Limitations

Bacterial growth rate from blood cultures and endocervical swabs were lower because 84.5% of the women had received broad spectrum antibiotics prior to specimen collection. However, this demonstrates efficient implementation of the policy of early administration of antibiotics upon recognition of severe sepsis. Media with resins, lytic agents, or other neutralizing substances would have increased blood culture growth rates in women with prior exposure to antibiotics. Due to funding constraints, one 5ml specimen was collected instead of the recommended minimum of two sets (aerobic + anaerobic) of blood cultures, each of ≥10ml, taken from separate venipuncture sites (42). I was unable to repeat blood cultures in women when *Bacillus sp* and *Coagulase negative staphylococci* were isolated to confirm if they were significant infections or contaminants. No strict anaerobes were isolated from both endocervical swabs and blood cultures because Amies transport media for endocervical swab is aerobic and may have inhibited anaerobic growth and no anaerobic blood culture bottle was collected in this study. Neisseria gonorrhea and Chlamydia trachomatis were not detected. This is attributed to the fastidious nature of the organisms which would have been better identified using nucleic acid amplification tests. Microbial susceptibility to ampicillin was not tested. Penicillin G, which has a narrower antimicrobial spectrum compared to ampicillin, was the only penicillin available and thus tested. Therefore antimicrobial resistance to penicillins may have been falsely elevated. Despite the funding constraints, this study provides important information on the microbiology of puerperal sepsis and the need for clinical culture surveillance.

The sample size may have been too small to detect significant differences during comparisons of microbiology with clinical outcomes among HIV infected and uninfected women.

5.3 Conclusion and Recommendations

The majority of women with puerperal sepsis have delivered by caesarian section. *E. coli* is the commonest isolate from the genital tract and blood stream, reflecting ascend of infection from the genitourinary tract and gastrointestinal system. There is emergence of MDRO gram negative bacilli resistant to carbapenems, especially *K. pneumoniae*. HIV infection is a risk factor for puerperal sepsis. MDRO and HIV-associated severe immunosuppression are independent risk factors for a higher disease severity score, need for surgery and prolonged hospital stay. *Enterobacter sp*. may be more pathogenic in HIV infection. Manual removal of the placenta may increase risk of bacteremia.

In the United Kingdom, the mortality rate from genital tract sepsis reduced by more than half between the trienniums 2006–08 and 2010–12 (1.13 per 100 000 maternities to 0.50 per 100 000) maternities) (13,29). A downward trend was maintained through to 2013 (0.29 per 100 000 maternities) (6). Initiatives such as the 'Surviving sepsis campaign and development of guidelines for recognition and management of severe sepsis have been largely responsible for this decrease (13). Prevention of community acquired and nosocomial multidrug resistant infections can be achieved through implementation of robust infection control strategies, emphasis on rational drug use, clinical culture surveillance to identify MDRO and monitoring of epidemiologic trends (66). More funding should be availed regularly for continuous education on infection control and to allow clinical culture surveillance and further research.

REFERENCES

1. Dolea C, Stein C. Global burden of maternal sepsis in the year 2000. 2003 [cited 2016 Jan 20]; Available from: http://www.who.int/entity/healthinfo/statistics/bod_maternalsepsis.pdf

2. World Health Organization, International Confederation of Midwives, editors. Education material for teachers of midwifery: midwifery education modules. 2nd ed. Geneva [Switzerland]: World Health Organization : International Confederation of Midwives; 2008. 6 p.

3. 3. Khalid S Khan, Daniel Woojdyla, Lale Say, A Metin Gulmezoglu, Paul F A Van Look. WHO Analysis of the Causes of Maternal Death: A Systematic Review. The Lancet. 2006 Apr 1;367(9516):1066-74. - Google Search [Internet]. [cited 2016 Jan 20].

4. Munjanja Steve P. Zimbabwe Maternal and Perinatal Mortality Study. Minist Health Child Welf Zimb. 2007;

5. Manjeya Farikai. Trends in Maternal Mortality for the Public Maternity Institutions of Harare, Zimbabwe, 2010 to 2014. University of Zimbabwe, Masters of Medicine in Obstetrics and Gynaecology; 2015.

6. Knight M, Kenyon S, Brocklehurst P, Neilson J, Shakespeare J, Kurinczuk JJ. on behalf of MBRRACE-UK. Saving Lives, Improving Mothers' Care-Lessons learned to inform future maternity care from the UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2009–12. Oxford: National Perinatal Epidemiology Unit, University of Oxford; 2014.

7. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap L, Wenstrom KD. Williams Obstetrics. 22nd ed. Vol. chapter 31. 2005.

8. Williams KL, Pastorek II JG. Postpartum endomyometritis. Infect Dis Obstet Gynecol. 1995;3(5):210–216.

9. Calvert C, Ronsmans C. HIV and the Risk of Direct Obstetric Complications: A Systematic Review and Meta-Analysis. PLoS ONE. 2013 Oct 4;8(10):e74848.

10. Zvandasara P, Saungweme G, Mlambo JT, Moyo J. Post Caesarean section infective morbidity in HIV-positive women at a tertiary training hospital in Zimbabwe. Cent Afr J Med. 2007;53(9–12):43–6.

11. Zvandasara P, Saungweme G, Mlambo JT, Moyo J. Post Caesarean section infective morbidity in HIV-positive women at a tertiary training hospital in Zimbabwe. Cent Afr J Med. 2007 Dec;53(9–12):43–7.

12. Sepsis following Pregnancy, Bacterial (Green-top Guideline No. 64b) [Internet]. Royal College of Obstetricians & Gynaecologists. [cited 2016 Jan 20]. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg64b/

13. Knight M, Kenyon S, Brocklehurst P, Neilson J, Shakespeare J, Kurinczuk JJE, et al. Saving Lives, Improving Mothers' Care Lessons learned to inform future maternity care from the UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity 2009-2012. 2014 [cited 2016 Jan 20]; Available from: http://discovery.ucl.ac.uk/1464330/

14. Bhanap PL SA. A clinical Study of puerperal sepsis. Glob Res Anal. 2013 Sep;2(9).

15. Bako B, Audu BM, Lawan ZM, Umar JB. Risk factors and microbial isolates of puerperal sepsis at the University of Maiduguri Teaching Hospital, Maiduguri, North-eastern Nigeria. Arch Gynecol Obstet. 2012 Apr;285(4):913–7.

16. Ahmed S, Hossain MA, Musa AK, Shamsuzzaman AK, Mahmud MC, Nahar K, et al. Preliminary report on anaerobic culture at Mymensingh Medical College Hospital in Bangladesh. Mymensingh Med J MMJ. 2010 Jan;19(1):10–5.

17. Gerstner G, Leodolter S, Rotter M. [Endometrial bacteriology in puerperal infections (author's transl)]. Z Für Geburtshilfe Perinatol. 1981 Oct;185(5):276–9.

18. Bako B, Ibrahim UN, Umar JB, Zamo AB. Microbial Isolates in Puerperal Sepsis and Their in vitro Antibiotic Sensitivity in North Eastern Nigeria. J Women's Health Care [Internet]. 2012;1(107). Available from: http://www.omicsgroup.org/journals/microbial-isolates-in-puerperal-sepsis-and-their-in-vitro-antibiotic-sensitivity-in-north-eastern-nigeria-2167-0420.1000107.php?aid=6325

19. World Health Organization, editor. The evolving threat of antimicrobial resistance: options for action. Geneva, Switzerland: World Health Organization; 2012. 119 p.

20. EDLIZ 2011 - 6th Essential Medicines List and Standard Treatment Guidelines for Zimbabwe [Internet]. [cited 2016 Jan 20]. Available from: http://apps.who.int/medicinedocs/en/d/Js21753en/

21. Mason PR, Katzenstein DA, Chimbira TH, Mtimavalye L. Vaginal flora of women admitted to hospital with signs of sepsis following normal delivery, cesarean section or abortion. The Puerperal Sepsis Study Group. Cent Afr J Med. 1989 Mar;35(3):344–51.

22. Mason PR, Gwanzura L, Latif AS, Ray S, Wijgert J, Katzenstein DA. Antimicrobial susceptibility patterns amongst group B streptococci from women in Harare, Zimbabwe. Int J Antimicrob Agents. 1996 May;7(1):29–32.

23. Essential Guide to Management of Common Obstetric and Gynaecology conditions in Zimbabwe. University of Zimbabwe, Department of Obstetrics and Gynaecology; 2012.

24. Sobel JD. Gynecologic Infections in Human Immunodeficiency Virus-Infected Women. Clin Infect Dis. 2000 Nov 15;31(5):1225–33.

25. Kamenga MC, De Cock KM, St Louis ME, Touré CK, Zakaria S, N'gbichi JM, et al. The impact of human immunodeficiency virus infection on pelvic inflammatory disease: a case-control study in Abidjan, Ivory Coast. Am J Obstet Gynecol. 1995 Mar;172(3):919–25.

26. Barbosa C, Macasaet M, Brockmann S, Sierra MF, Xia Z, Duerr A. Pelvic inflammatory disease and human immunodeficiency virus infection. Obstet Gynecol. 1997 Jan;89(1):65–70.

27. Korn AP, Landers DV, Green JR, Sweet RL. Pelvic inflammatory disease in human immunodeficiency virus-infected women. Obstet Gynecol. 1993 Nov;82(5):765–8.

28. Palaniappan N, Menezes M, Willson P. Group A streptococcal puerperal sepsis: management and prevention. Obstet Gynaecol. 2012 Jan 1;14(1):9–16.

29. Cantwell R, Clutton-Brock T, Cooper G, Dawson A, Drife J, Garrod D, et al. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. BJOG Int J Obstet Gynaecol. 2011 Mar;118 Suppl 1:1–203.

30. Bosch J, Pericot A, Amorós M, Ros R. [Puerperal endometritis: study of 52 clinically and microbiologically diagnosed cases]. Enfermedades Infecc Microbiol Clínica. 1995 Apr;13(4):203–8.

31. Temmerman M, Chomba EN, Ndinya-Achola J, Plummer FA, Coppens M, Piot P. Maternal human immunodeficiency virus-1 infection and pregnancy outcome. Obstet Gynecol. 1994 Apr;83(4):495–501.

32. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268–81.

33. McGowan JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. Am J Med. 2006 Jun;119(6 Suppl 1):S29-36-70.

34. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. Clin Infect Dis Off Publ Infect Dis Soc Am. 2006 Sep 1;43 Suppl 2:S49-56.

35. Pitout JDD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008 Mar;8(3):159–66.

36. Mason PR, Gwanzura L, Chimbira TH, Mtimavalye L, Nathoo J. Antimicrobial susceptibility of Neisseria gonorrhoeae isolated from maternal and child infections in Harare. The Puerperal Sepsis Study Group. Cent Afr J Med. 1989 Apr;35(4):367–71.

37. Mutimutema M. To determine the prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus (MRSA) in the hospital and burns units. Unpubl Artic.

38. World Health Organisation. Opportunities for Africa's Newborns: Practical data, policy and programmatic support for newborn care in Africa. 2006;

39. Managing Prolonged and Obstructed Labor [Internet]. [cited 2016 Jan 20]. Available from: http://apps.who.int/iris/bitstream/10665/44145/4/9789241546669_4_eng.pdf

40. WHO recommendations for the Prevention and Treatment of Postpartum Hemorrhage [Internet]. [cited 2016 Jan 20]. Available from: http://apps.who.int/iris/bitstream/10665/75411/1/9789241548502_eng.pdf

41. WHO :: Global Database on Body Mass Index [Internet]. [cited 2016 Jan 20]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html

42. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med. 2008 Jan;36(1):296–327.

43. Blood Transfusions in Obstetrics (Green-top Guideline No. 47) [Internet]. Royal College of Obstetricians & Gynaecologists. [cited 2016 Jan 20]. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg47/

44. KI_SuppCover_2.1.indd - KDIGO AKI Guideline.pdf [Internet]. [cited 2016 Jan 20]. Available from: http://www.kdigo.org/clinical_practice_guidelines/pdf/KDIGO%20AKI%20Guideline.pdf

45. Interim WHO Clinical Staging of HIV/AIDS and HIV/AIDS case definitions for surveillance African Region 2005 [Internet]. [cited 2016 Jan 20]. Available from: http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf?ua=1

46. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, 2nd Edition. 2 edition. Cambridge ; New York: Cambridge University Press; 2006. 440 p.

47. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. 2011;32(1).

48. Weber DJ, Saviteer SM, Rutala WA, Thomann CA. Clinical significance of Bacillus species isolated from blood cultures. South Med J. 1989 Jun;82(6):705–9.

49. Cotton DJ, Gill VJ, Marshall DJ, Gress J, Thaler M, Pizzo PA. Clinical features and therapeutic interventions in 17 cases of Bacillus bacteremia in an immunosuppressed patient population. J Clin Microbiol. 1987 Apr;25(4):672–4.

50. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, et al. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis Off Publ Infect Dis Soc Am. 1997 Apr;24(4):584–602.

51. Smaill F, Hofmeyr GJ. Antibiotic prophylaxis for cesarean section. Cochrane Database Syst Rev. 2002;(3):CD000933.

52. Acosta CD, Kurinczuk JJ, Lucas DN, Tuffnell DJ, Sellers S, Knight M, et al. Severe Maternal Sepsis in the UK, 2011–2012: A National Case-Control Study. Fisk NM, editor. PLoS Med. 2014 Jul 8;11(7):e1001672.

53. Souvenir D, Anderson DE, Palpant S, Mroch H, Askin S, Anderson J, et al. Blood Cultures Positive for Coagulase-Negative Staphylococci: Antisepsis, Pseudobacteremia, and Therapy of Patients. J Clin Microbiol. 1998 Jul 1;36(7):1923–6.

54. Guiral E, Bosch J, Vila J, Soto SM. Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiol Lett. 2011 Jan;314(2):170–3.

55. Brink A, Feldman C, Richards G, Moolman J, Senekal M. Emergence of extensive drug resistance (XDR) among Gram-negative bacilli in South Africa looms nearer. SAMJ South Afr Med J. 2008;98(8):586–592.

56. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013 Dec;13(12):1057–98.

57. Stone PW, Gupta A, Loughrey M, Della-Latta P, Cimiotti J, Larson E, et al. Attributable costs and length of stay of an extended-spectrum beta-lactamase-producing Klebsiella pneumoniae outbreak in a neonatal intensive care unit. Infect Control Hosp Epidemiol. 2003 Aug;24(8):601–6.

58. Butler AM, Olsen MA, Merz LR, Guth RM, Woeltje KF, Camins BC, et al. Attributable costs of enterococcal bloodstream infections in a nonsurgical hospital cohort. Infect Control Hosp Epidemiol. 2010 Jan;31(1):28–35.

59. Cosgrove SE. The Relationship Between Antimicrobial Resistance and Patient Outcomes: Mortality, Length of Hospital Stay, and Health Care Costs. Clin Infect Dis Off Publ Infect Dis Soc Am. 2006;42 Suppl 2(Suppl 2):S82-9.

60. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrob Agents Chemother. 2006 Jan;50(1):43–8.

61. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Meta-analysis. Clin Infect Dis. 2003 Jan 1;36(1):53–9.

62. Kang C-I, Kim S-H, Park WB, Lee K-D, Kim H-B, Oh M, et al. Bloodstream Infections Caused by Enterobacter Species: Predictors of 30-Day Mortality Rate and Impact of Broad-Spectrum Cephalosporin Resistance on Outcome. Clin Infect Dis. 2004 Sep 15;39(6):812–8.

63. Zimbabwe Demographic and Health Survey 2010-11 - FR254.pdf [Internet]. [cited 2016 Jan 21]. Available from: http://www.dhsprogram.com/pubs/pdf/FR254/FR254.pdf

64. Manfredi R, Nanetti A, Ferri M, Chiodo F. Enterobacter spp. Infections Complicating the Course of HIV Disease. J Chemother. 2001 Jan 1;13(2):195–201.

65. Cohen CR, Sinei S, Reilly M, Bukusi E, Eschenbach D, Holmes KK, et al. Effect of human immunodeficiency virus type 1 infection upon acute salpingitis: a laparoscopic study. J Infect Dis. 1998;178(5):1352–1358.

66. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Committee HICPA, others. Management of multidrug-resistant organisms in health care settings, 2006. Am J Infect Control. 2007;35(10):S165–S193.

APPENDIX 1

Table a: Endocervical swab isolates

Endocervical swab isolates	**Number of isolates**	**% of total isolates**
E. coli	38	30.6
K. pneumonia	19	15.3
CoNS	9	7.3
S. aureus	8	6.5
Group D streptococcus	8	6.5
P. aeruginosa	5	4.0
Enterobacter sp	5	4.0
Corynebacterium sp	5	4.0
S. pyogenes	4	3.2
C. freundii	4	3.2
Alcaligenes sp	3	2.4
M. morganii	2	1.6
Providencia sp	2	1.6
S. viridans	2	1.6
Shigella sp	2	1.6
Yersinia sp	2	1.6
Bacillus sp	2	1.6
S. agalactae	1	0.8
Salmonella sp	1	0.8
Kluyvera sp	1	0.8
K. oxytoca	1	0.8
Total number of isolates	124	100.0

Table b: Blood culture isolates

Blood culture isolates	Day 1	Day 5	Day 10	Overall	% of total isolates (n=14)
E. coli	2	0	0	2	14.3
Bacillus sp	1	1	3	5	35.7
Coagulase negative staphylococcus	0	2	1	3	21.4
S. aureus	0	1	0	1	7.1
Alcaligenes sp	0	0	1	1	7.1
Moraxella sp	0	1	0	1	7.1
Fungus	0	1	0	1	7.1
Total number of isolates	3	6	5	14	100.0

Table c: Antimicrobial susceptibility profiles of endocervical swab isolates

		CRO		P		C		Gn		Clin		CIP		E	
Isolate	**Susceptibilit**	N	%	N	%	N	%	N	%	N	%	N	%	N	%
E. coli	S	15	39.	1	2.6	23	60.	18	47.	1	2.6	25	65.	4	10.
	R	23	60.	36	94.	15	39.	19	50.	35	92.	13	34.	33	86.
	I	0	0	1	2.6	0	0	1	2.6	2	5.3	0	0	1	2.6
K. pneumonia	S	6	31.	2	10.	7	36.	7	36.	0	0	11	57.	0	0
	R	13	68.	17	89.	12	63.	12	63.	19	100	8	42.	18	94.
	I	0	0	0	0	0	0	0	0	0	0	0	0	1	5.3
K. oxytoca	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1	100	1	100	1	100	1	100	1	100	1	100	1	100
M. morganii	S	1	50.	0	0	1	50.	1	50.	0	0	1	50.	0	0
	R	1	50.	2	100	1	50.	1	50.	2	100	1	50.	2	100
P. aeruginosa	S	0	0	0	0	2	40.	4	80.	1	20.	3	60.	0	0
	R	5	100	5	100	3	60.	1	20.	4	80.	2	40.	5	100
Coagulase negative staphylococcus	S	4	44.	4	44.	5	55.	5	55.	9	100	6	66.	5	55.
	R	4	44.	5	55.	4	44.	3	33.	0	0	3	33.	4	44.
	I	1	11.	0	0	0	0	1	11.	0	0	0	0	0	0
S. aureus	S	4	50.	5	62.	5	62.	4	50.	4	50.	6	75.	4	50.
	R	4	50.	3	37.	2	25.	4	50.	4	50.	2	25.	4	50.
	I	0	0	0	0	1	12.	0	0	0	0	0	0	0	0
Group D Streptococcus	S	*		6	75.	4	50.	*		*		4	50.	1	12.
	R			2	25.	4	50.					3	37.	5	62.
	I			0	0	0	0					1	12.	2	25.
Providencia	S	0	0	0	0	2	100	0	0	0	0	0	0	0	0
	R	2	100	2	100	0	0	2	100	2	100	1	50.	2	100
	I	0	0	0	0	0	0	0	0	0	0	1	50.	0	0
Corynebacterium sp	S	4	80.	1	20.	2	40.	3	60.	1	20.	3	60.	3	60.
	R	1	20.	4	80.	3	60.	2	40.	4	80.	2	40.	2	40.
Enterobacter sp	S	1	20.	1	20.	3	60.	0	0	0	0	2	40.	0	0
	R	4	80.	4	80.	2	40.	5	100	5	100	2	40.	5	100
	I	0	0	0	0	0	0	0	0	0	0	1	10.	0	0
S. pyogenes	S	2	50.	4	100	2	50.	3	75.	2	50.	3	75.	2	50.
	R	2	50.	0	0	0	0	1	25.	0	0	1	25.	0	0
	I	0	0	0	0	2	50.	0	0	2	50.	0	0	2	50.
C.freundii	S	0	0	1	25.	2	50.	2	50.	1	25.	0	0	0	0
	R	4	100	3	75.	2	50.	2	50.	3	75.	4	100	4	100
Alcaligenes sp	S	0	0	0	0	0	0	2	66.	0	0	1	33.	0	0
	R	3	100	3	100	3	100	1	33.	3	100	2	66.	3	100
Shigella sp	S	1	50.	0	0	2	100	1	50.	0	0	1	50.	0	0
	R	1	50.	2	100	0	0	1	50.	2	100	1	50.	2	100
Yersinia sp	S	0	0	0	0	2	100	0	0	0	0	0	0	0	0
	R	2	100	2	100	0	0	2	100	2	100	1	50.	2	100
	I	0	0	0	0	0	0	0	0	0	0	1	50.	0	0
S. viridans	S	2	100	*		1	50.	*		2	100	*		2	100
	R	0	0			1	50.			0	0			0	0
S. agalactae	S	1		1		1		0		1		1		1	
	R	0		0		0		1		0		0		0	
Salmonella sp	S	0		0		1		1		0		1		0	
	R	1		1		0		0		1		0		1	
Kluyvera sp	S	0		0		1		0		0		0		0	
	R	1		1		0		1		1		1		1	

Key:

CRO- ceftriaxone, P- penicillin, C- chloramphenicol, Gn- gentamycin, Clin- clindamycin, CIP- ciprofloxacin, E- erythromycin

S- Susceptible
I- Intermediate sensitivity
R- Resistant
No- number
* on table or blank space on graphs- Susceptibility not tested including *Bacillus sp*. See section 3.7.3.4.d.

Table d: Multidrug resistant isolates

Isolate	**No of MDRO**	**Total isolates**	**% MDRO**
K. pneumonia	6	19	32%
E. Coli	2	38	5%
K. oxytoca	1	1	100%
M. morganii	1	2	50%
Enterobacter sp	1	5	20%
P. aeruginosa	1	5	20%
Alcaligenes sp	1	3	33%
C. freundii	1	4	25%
S. aureus	1	9	11%
Total	15		