FACTORS AND COMMON BACTERIAL PATHOGENS ASSOCIATED WITH POST-CAESAREAN WOUND SEPSIS AT HOIMA REGIONAL REFERRAL HOSPITAL, UGANDA

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DECLARATION

I, Muhumuza Ismael, hereby declare that this dissertation was a result of my own original work and that it has never been submitted to any other institution of higher learning locally or internationally for any award.

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APPROVAL

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DEDICATION

A great deal of time and effort has been incurred in the course of developing this book. I dedicate this work to my Father Mohammed Bituura Agaba, my dear Uncle Reverend Cannon Benon Byamugisha, my mother Kyampaire Rehema and to my supervisors, all lecturers and colleagues who serve in Obstetrics and Gynaecology department at Kampala International University Teaching Hospital.

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LIST OF ABBREVIATIONS AND ACRONYMS

APH	Antepartum Hemorrhage
E. *coli*	*Escherichia coli*
HRRH	Hoima Regional Referral Hospital
KIU-REC	Kampala International University Research Ethics Committee
KIU-WC	Kampala International University- Western Campus
MRSA	Methicillin-Resistant *Staphylococcus aureus*
PCT	Procalcitonin
PET	Pre-eclamptic Toxemia (Preeclampsia)
RCOG	Royal College of Obstetrics and Gynecology
BMC	BioMed Central
BMI	Body Mass Index
LTCS	Lower Transverse Caesarean Section
KNH	Kenyatta National Hospital
CS	Caesarean Section
cOR	crude Odds Ratio
aOR	adjusted Odds Ratio
CI	Confidence Interval
Sd	Standard Deviation
IQR	Interquartile Range

OPERATIONAL DEFINITIONS

Antibacterial agent: A drug that kills bacteria or stops their growth (Matinyi *et al.*, 2018).

Antibiotic prophylaxis: Antimicrobial drug administered in absence of any signs or symptoms of sepsis to prevent occurrence of sepsis (Dhar *et al.*, 2014).

Caesarean section: The delivery of a baby, placenta and membranes through a surgical incision in the mother's abdominal wall and uterine after 28 weeks of amenorrhea (Chu *et al.*, 2012).

Immediate puerperium: The first twenty four hours following termination of pregnancy.

Puerperium: Period from the termination of labor to complete involution of the uterus, usually defined as forty two days (RCOG, 2012).

Post-caesarean wound sepsis: Infection that develops on the incision site following caesarean delivery and is diagnosed by clinician (Gelaw *etal.*, 2017).

Prolonged rupture of Membranes: The rupture of membranes for more than 24 hours before onset of labor (Barros *et al.*, 2010).

Premature rupture of Membranes: Rupture of membranes at least one hour before onset of labor

Sepsis: A condition that is life-threatening which occurs when the body's response to infection causes injury to its own organs and tissues (Kabau, 2014).

Wound: Trauma to living tissue caused by a blow or cut resulting into a cut or breakage in skin (Gelaw *et al.*, 2017).

ABSTRACT

Background: Post-caesarean wound sepsis is among the most common problem for patients who undergo caesarean section. It remains a common and widespread problem contributing to morbidity and mortality; this could be due to an increase in antimicrobial resistant bacterial pathogens. Therefore, a study to identify and document the factors associated with wound sepsis and common bacterial pathogens can provide solution to prevent incidence and establish microbiological mapping, and this is the intension for this research.

Objectives: To determine prevalence, identify factors, common bacterial pathogens from post-caesarean wounds and antibacterial susceptibility pattern at Hoima Regional Referral Hospital.

Research methods: This was a cross-sectional study conducted among patients with post-caesarean wound sepsis in the post-natal wards at Hoima Regional Referral Hospital. Consecutive enrolment of 303 participants who consented to participate was done daily until a required sample size was realized from July to September, 2018. Structured questionnaires were used to collect data on associated factors and wound swabs were done. Culturing for colony characteristics followed by Gram stain was used for provisional identity of pathogenic bacteria. Further identification was done by a set of biochemical tests. Antibacterial susceptibility pattern of isolated bacterial pathogens was determined by Kirby Bauer disc diffusion method. Data was analyzed using STATA VERSION 14.2.

Results: The wound sepsis rate was 16.8%. Being educated, multiple vaginal examination, hygiene, previous caesarean sections and HIV seropositivity were all significantly associated with wound sepsis (P value <0.05). Majority of the wound swab specimen yielded *Staphylococcus aureus*, and the least-prevalent pathogen was *Proteus mirabilis*. Coliforms showed high susceptibility to Imipenem followed by *Staphylocccocus aureus* to ciprofloxacin. Resistance was highest for coliforms and *Staphylococcus aureus* against, ciprofloxacin, gentamycin, ceftriaxone and cotrimoxazole.

Conclusions and recommendations: The rate of caesarean wound sepsis is high at Hoima Regional Referral Hospital. Being educated, multiple vaginal examination, hygiene, previous caesarean sections and HIV sero-positivity are significantly associated with wound sepsis. *Staphylococcus aureus* is the commonest organism isolated from exudates of septic wounds after caesarean section. Health workers should be informed about the high prevalence, the identified associated factors and common pathogens for proper management and also rational use of antibiotics to combat resistance.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background to the study

Caesarean section is delivery of a baby through a surgical incision in the mother's abdomen and uterus after 28 weeks of amenorrhea (Chu *et al.*, 2012). Caesarean section is done when vaginal delivery may cause a risk to the mother or baby such as when there is fetal distress, or when the baby is abnormally positioned or there is prolonged labor or a case of maternal factors such as pre-eclampsia or contracted pelvis.

Sepsis is a life-threatening illness caused by the body's response to an infection and develops when mediators of inflammation are released in the general body circulation (Prucha, Bellingan, & Zazula, 2015; Singer *et al.*, 2016). Modern understanding of sepsis is in reference to human response to infection and this is mediated by several inflammatory mediators (Vincent *et al.*, 2013). This shows that sepsis is a complex physiological and metabolic response of the body and is currently a major reason for admission of patients to the intensive care unit (Angus & van der Poll, 2013).

In medical practice, post-operative sepsis has been recognized as a major cause of mortality and morbidity in patients as a result of dysregulation of host immune response to infection. This leads to production of cytokines, prostanoids and nitric oxide, which suppress immunity of the body further (Monkhouse, 2006). In addition, the leading cause of severe sepsis has been shown to be related primarily to Gram-negative bacterial infections and the prognosis is grave in older people, black race and those with a pre-existing illness (Mayr *et al*,. 2014). In addition, gram positive bacteria, in particular *Streptococci* species have also been isolated as major pathogens in sepsis (Acosta & Knight, 2013).

Post-caesarean wound sepsis is common among women following caesarean section and this can easily progress into septic shock if poorly managed (Kalisa, Rulisa, van den Akker, & van Roosmalen, 2016; Morgan & Roberts, 2013; Sagy, Al-Qaqaa, & Kim, 2013). Early diagnosis of post-caesarean wound sepsis using sepsis biomarkers (Omar, 2010) such as pro-calcitonin (PCT) is an important medical practice. These biomarkers are hardly available in several health care units in developing countries and this has led to the development of a global campaign on development of appropriate guidelines for successful management of sepsis (Bloos & Reinhart, 2014; Dellinger *et al.*, 2013). Successful management is crucial to

ensure that unnecessary prolonged activation of the immune response is inhibited (Prucha *et al.*, 2015; Stearns-Kurosawa *et al.*, 2011; Yealy *et al.*, 2015).

Though caesarean sections are done under aseptic conditions, the risk of post-caesarean wound sepsis always exists (Dlamini *et al.*, 2015), which puts post-caesarean wound sepsis among the most common nosocomial infections. Globally, the rate of caesarean section is increasing rapidly (Ostovar *et al.*, 2010) and the most common complication after caesarean section is wound sepsis, with an incidence of 3-15% (Zuarez-Easton *et al,*. 2017a). Post-caesarean wound sepsis is also associated with maternal mortality rate of up to 3% especially in health units that have no facilities to carryout safe caesarean sections or treat post-caesarean complications (Gibbons *et al.*, 2012, Zuarez-Easton *et al,*. 2017b).

In sub-Saharan Africa, post-caesarean wound sepsis has been shown to be in the range of 1.7% to 10.4% showing that the condition is significant in the continent (Chu *et al,*. 2015; Harrison & Goldenberg, 2016). This has been attributed to poor accessibility to health care services which is below 3% and thus resulting in poor postnatal follow up and wound care (Chu *et al.*, 2012; Irani & Deering, 2015). In addition, post-caesarean wound sepsis in sub Saharan Africa has been associated with poverty, environmental pollution, poor preoperative care, malnutrition, anemia, wound contamination, poor antibiotic selection and poor immunity (Gelaw *et al.*, 2017).

In East Africa, the rate of caesarean sections is below 40%, which is a higher rate (Worjoloh *et al.*, 2012) compared to the recommended rate of caesarean section as per the WHO which considers that the best caesarean section rate is between 10-15% (Uriel, 2018). This increases the risk of post-caesarean wound sepsis.

In Rwanda the prevalence of post-caesarean wound sepsis has been shown to be at 4.9% (Bizimana *et al.*, 2016) and in a parallel study from Zanzibar, post-caesarean wound sepsis was shown to be caused primarily by Staphylococcus aureus and Escherichia coli and these had led to increased hospital stay (Omar, 2010).

In Uganda, a study conducted in Jinja (Anguzu, 2007) showed that the major pathogenic bacteria associated with post-operative sepsis are; *Staphylococcus aureus,* coliforms, *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter spp.* In addition, the study showed that these pathogens were highly resistant to ampicillin, amoxicillin and chloramphenicol. The threat of post-operative bacterial sepsis in HIV

infected patients has been established at 0.9% and majority of bacterial isolates have been reported to be resistant to common antibiotics used (Anguzu & Olila, 2007; Kateete *et al*,.2011; Sekirime & Lule, 2009).

A study conducted at Mulago National Referral Hospital in Uganda showed that majority of these septic wounds have pathogens that are methicillin resistant thus posing a major healthcare challenge due to the limited class of drugs available in several healthcare centers in Uganda (Kateete *et al*., 2011). A more recent study has shown antimicrobial resistance of in 80% of post-partum mothers in Uganda (Bebell *et al*,. 2017).

At Hoima Regional Referral Hospital (HRRH) there was a high turnover of patients with eight to ten caesarean sections done in a day (maternity theatre register). It was also noted that theatres were being shared by other surgical teams and that the patients with post-caesarean wound sepsis were not isolated from the rest of the patients in ward, according to the findings during an onsite visit to the Hospital.

Several associated factors including patient related factors, hospital factors and obstetric factors have made management of post-caesarean wound sepsis challenging, leading to an increase in hospital stays (Dhar *et al*., 2014). Hence, thorough identification of the bacterial pathogens and the associated factors of post-caesarean wound sepsis is important for developing proper protocols to reduce its incidence and complications.

1.2 Problem statement

According to semi-annual report of 2017, Hoima Regional Referral Hospital (HHRH) receives 20-30 patients in labor per day including 7-10 referrals, with a minimum of ten caesarean deliveries being done per day (HRRH semi-annual maternal report, 2017). Post-caesarean wound sepsis in HRRH is high despite using aseptic technique and department protocol of intravenous triple antibiotics (Ceftriaxone, Metronidazole and Gentamycin) after caesarean section.

According to HRRH maternal report, in the month of September, 2017, there were 33 cases of wound sepsis out of 199 caesarean sections done, which translates to a rate of 16.6%. This leads to prolonged hospital stays and increased risk of complications like burst abdomen, hysterectomy, repeat operations and even maternal death. This not only burdens the health system greatly but it also financially depletes the patients and their families. The factors

behind this high rate of caesarean wound sepsis, associated pathogens and their susceptibility patterns have not been studied at HRRH.

1.3 Objectives

1.3.1 Purpose of the study

To assess the factors and to identify common bacterial pathogens associated with post-caesarean wound sepsis at Hoima Regional Referral Hospital.

1.3.2 Specific objectives

1. To determine the prevalence of post-caesarean wound sepsis at Hoima Regional Referral Hospital.
2. To identify the factors associated with post-caesarean wound sepsis at Hoima Regional Referral Hospital.
3. To identify the common bacterial pathogens among patients with post-caesarean wound sepsis patients at Hoima Regional Referral Hospital.
4. To assess the antibacterial drug susceptibility pattern of bacterial isolates among patients with post-caesarean wound sepsis at Hoima Regional Referral Hospital.

1.4 Research questions

1. What is the prevalence of post-caesarean wound sepsis at Hoima Regional Referral Hospital?
2. What are the factors associated with post-caesarean wound sepsis at Hoima Regional Referral Hospital?
3. What are the common bacterial pathogens among patients with post-caesarean wound sepsis at Hoima Regional Referral Hospital?
4. What is the antibacterial drug susceptibility pattern of bacterial isolates among patients with post-caesarean wound sepsis at Hoima Regional Referral Hospital.?

1.5 Justification

In Uganda, a couple of studies have been conducted on burns and post-operative sepsis (Anguzu, 2007; Kateete *et al.*, 2011), while limited studies have been conducted primarily on caesarean section patients. In addition, a high percentage (80%) of antimicrobial resistance

has been reported (Bebell *et al.*, 2017) which implies that the management of post-caesarean section mothers may be complicated. Information gathered from this study would help to reduce the incidence of post-caesarean wound sepsis, improve prognosis in affected patients, and develop effective strategies for a reduction in antimicrobial resistance through effective therapy administration. This would subsequently lead to establishment of microbiological mapping at Hoima Regional Referral Hospital.

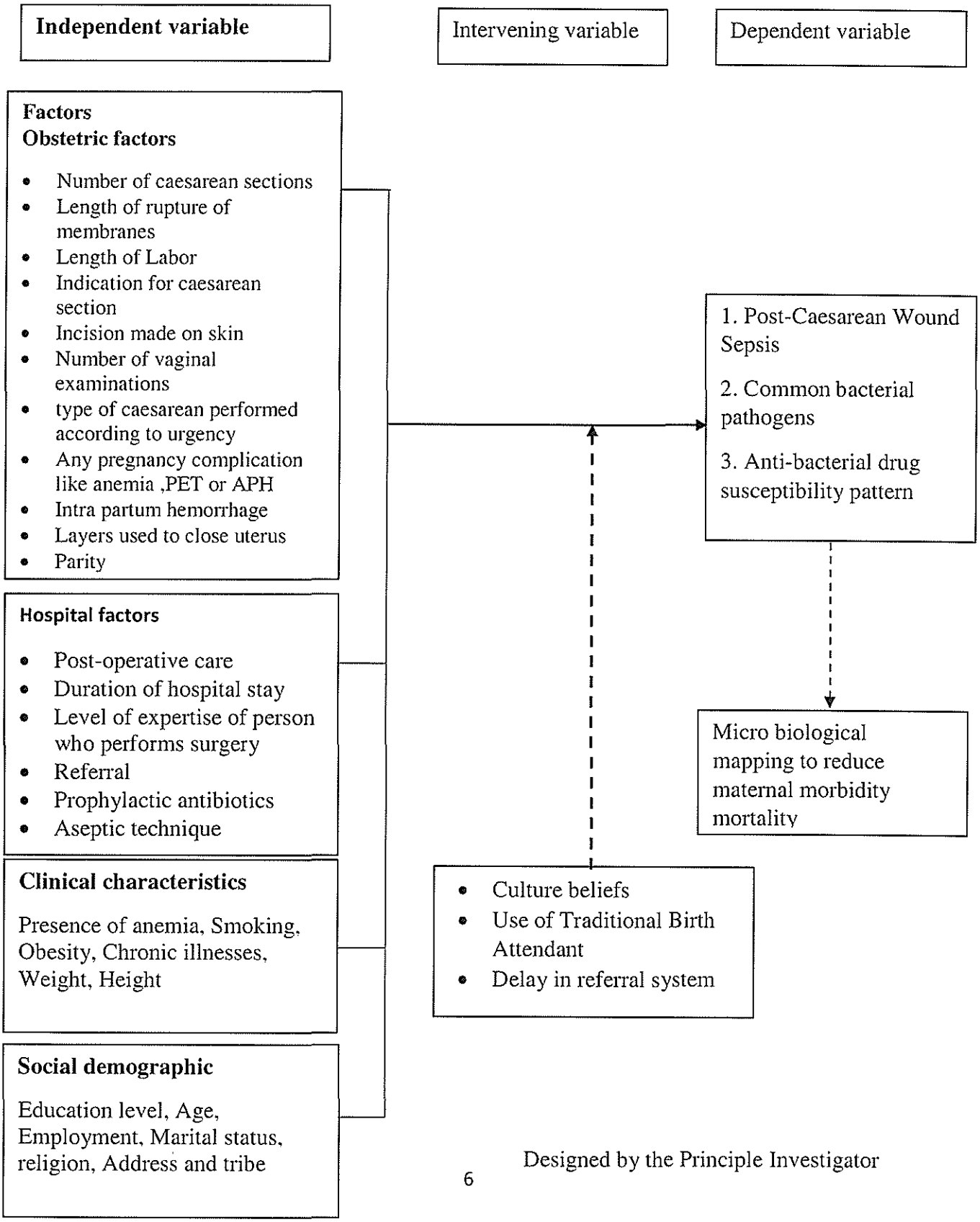
1.6 Significance of the study

The ministry of health and research institutions will gain information on the common bacterial isolates in post-caesarean wound sepsis (Omar, 2010; Royal College of Obstericians & Gynaecologists, 2012), thus helping in formulating appropriate policies for improved health service provision in the region and increase availability of the drugs sensitive to the common bacteria. This may subsequently lead to improved livelihoods in the community and save the government and patients from costs that would be spent treating resistant microbial agents (Dellinger *et al.*, 2013; Morgan & Roberts, 2013).

The study findings established the current rates of post-caesarean wound sepsis at Hoima Regional Referral Hospital. Since the study assessed the various factors associated with post-caesarean wound sepsis, the results may form a basis for future measures to reduce post-caesarean wound sepsis at the Hoima Regional Referral Hospital in addition to serving as a benchmark for future references for post-caesarean wound sepsis.

1.7 Conceptual framework

Figure 1: Conceptual framework



Designed by the Principle Investigator

1.7.1 Description of Conceptual Framework

The diagram above shows the interaction between the independent (obstetric, hospital factors, clinical characteristics and social demographic), intervening (use of traditional birth attendants and delay in referral system) and dependent variables (Post-ceasarean wound Sepsis, Common bacterial pathogens and Anti-bacterial drug susceptibility pattern) and in turn reduce maternal morbidity and mortality.

Factors that might affect the woman following caesarean section to get post-caesarean wound sepsis include: Obstetric factors for example parity; prolonged labor; indication for caesarean section like obstructed labor; length of labor; number of caesarean sections; wrong technique of caesarean section; type of caesarean section performed according to urgency (emergency or elective caesarean section); incision made on the skin; layers used to close the uterus; any pregnancy complication like APH; Post-partum hemorrhage; PET; and number of vaginal examinations, might increase the chance of introducing bacteria into the uterus through the vagina.

Hospital factors for example post-operative care, duration of hospital stay, level of expertise of person who performs the surgery, referral, prophylactic antibiotics, aseptic technique used, and clinical characteristics for example presence of anemia, smoking, obesity, and chronic illnesses like diabetes, hypertension among others, may contribute to the development of wound sepsis after caesarean section. Others that contribute to this may be socio-demographic factors for example low socioeconomic status, hygiene, marital status, religion, education and age.

1.8 Study scope

1.8.1 Content Scope

The study placed emphasis on factors and common bacterial pathogens associated with prevalence of post-caesarean wound sepsis. Women with eligibility criteria were recruited and consented. Exudate swabs were collected to determine bacterial causes of post-caesarean wound sepsis. In particular, emphasis was placed on Gram positive and Gram negative bacterial pathogens and also their drug susceptibility testing antibiotic discs: Gentamycin, Ceftriaxone, ciprofloxacin, ampicillin, amoxiclav, cotrimoxazole, chloramphenicol, tetracycline, imipenem, vancomycin and penicillin. A questionnaire was filled to obtain the factors associated with post-caesarean wound sepsis.

1.8.2 Geographical Scope

The study was done at Hoima Regional Referral Hospital in the maternity postnatal ward. Mothers who meet eligibility criteria were recruited from the postnatal ward and a private side room allocated for the study was used. The study participants came from catchment areas of Hoima Regional Referral Hospital such as Kiboga, Mubende, Masindi, Kiryandongo and other neighboring districts.

1.8.3 Time Scope

The data collection period was limited to a period of July 2018 to September 2018. This was adequate duration to achieve the study sample size basing on the reports of admissions at HRRH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prevalence of post-caesarean wound sepsis

Compared to spontaneous vaginal delivery, caesarean section is associated with increased neonatal and maternal morbidity and mortality (Oboro *et al.*, 2010). Post-caesarean delivery complications include wound separation and once complicated by infections may develop into sepsis (Quinlan & Murphy, 2015), thus showing a need to identify the antibacterial sensitivity profile in the bacterial isolates for effective patient management. Post-caesarean wound sepsis represent a significant health and economic challenge and identifying the organisms and techniques to manage caesarean wounds is essential for obstetricians (Fitzwater & Tita, 2014).

Following invasion of the wound by pathogenic bacteria and mismanagement, wound sepsis has been found to be an inevitable outcome (Singer *et al.*, 2016). Sepsis is a condition that is life-threatening and it occurs when the body's response to infection causes injury to its own organs and tissues. Since this high prevalence is in spite of the advances in medical knowledge and treatment (Royal College of Obstericians & Gynaecologists, 2012), it demonstrates a need to understand it better for its effective management. Moreover caesarean delivery remains one of the most important factors of puerperal sepsis of which post-caesarean wound sepsis is among (Conroy *et al.*, 2012) since mothers undergoing caesarean section have a 5 to 20-fold higher chance of getting puerperal sepsis compared with mothers who give birth vaginally (Kabau, 2014).

Post-caesarean wound sepsis remains a major source of morbidity and mortality in postpartum mothers especially in the developing countries like Uganda (Kabau, 2014). Post-caesarean wound sepsis is also associated with long duration of hospital stay, increased cost of care and increased morbidity and mortality (Dhar *et al.*, 2014).

There are several factors that exacerbate post-caesarean wound sepsis including patient related factors, hospital factors and obstetric factors. It is these factors that have made management of post-caesarean wound sepsis challenging and increase in hospital stays (Dhar *et al.*, 2014) Hence, thorough identification of the bacterial pathogens and factors that are associated with post-caesarean wound sepsis is important for developing proper protocols to reduce its incidence and complications.

In one study by Ngowe *et al.*, (2014), they linked the high prevalence of post-operative infection (20.6%) to the fact that most of their participants were post-caesarean section patients. They reported that the surgical site after a caesarean section is more prone to infection given that there is a direct connection between the site and the bacteria flora environment of the vagina. Besides, before a caesarean section, the patient is subjected to multiple vagina/cervical examinations which carry bacteria from the vagina into the bacteria-free uterine cavity. The rate of 19.4% of post-caesarean wound sepsis was similar to 7 – 20% reported by (Ngowe *et al.*, 2014).

In another study, one in ten (11%) of all caesarean sections had developed wound sepsis. The figure might have been largely underestimated as the study was exclusively reliant on medical records review and it did not involve post-discharge follow-up. Further studies conducted in Norway and Scotland reported that 86% (15) and 71% (16) of the sepsis occurred after discharge and also the most of the diagnosis was based on clinical basis and could have missed the patients who may not present with the classical signs of inflammation (Wodajo, Belayneh, & Gebremedhin, 2017).

2.2 Factors associated with post-caesarean wound sepsis

In the development of post-caesarean wound sepsis, increased number of parity, presence of non-communicable diseases and infectious diseases , anaemia, pre-eclampsia and being obese are the major factors that have been associated with post-caesarean wound sepsis (Dhar *et al.*, 2014). In East Asia, the main factors for surgical site sepsis amongst post-operative mothers have been found to be independently related and these include pre-operative remote infection, chorioamnionitis, maternal preoperative condition especially among those with an ongoing infection, pre-eclampsia, higher body mass index (obesity) and increased blood loss during surgery (Dagshinjav *et al*,. 2017).

In addition, a study conducted at the Washington University Teaching Hospital by Temming *et al.*, (2017), showed that post-operative sepsis was common amongst mothers who had been subjected to a low transverse caesarean section technique and prophylactic treatment using cephalosporins was associated with improved outcomes. Also, according to Chu *et al.*, (2015), premature rupture of membranes and increased number of vaginal examinations were associated with post-caesarean wound sepsis. This has subsequently led to the need for making adjustments while dealing with patients who are at high risk of developing post-

caesarean wound sepsis. Consequently, the Cohen's incision for entry, single closure of the uterus and non-closure of both layers of the peritoneum have been recommended for effective management of high risk patients (Hema & Johanson, 2002).

Caesarean sections wound sepsis classification involves four classes, namely:- Class I which involves a clean wound in which no inflammation is encountered on entry; Class II which is a clean-contaminated wound in which entry is done under controlled conditions and basic level of contamination may be observed as per routine caesarean sections; Class III caesarean section which involves contamination in which there is an accidental major breakage in sterile technique or spillage from the uterine contents; and Class IV caesarean section wound sepsis which is common in old traumatic wounds with retained devitalized tissue, and which involves dirt and severe infection of the surgical wound. This is common among patients who are undertaking multiple caesarean sections and is complicated by pre-existing clinical infection (Conroy *et al.*, 2012).

In addition, more recent evidence has shown that using a plastic retractor instead of the traditional Collins metal self-retaining wound retractor, reduces the risk for the development of post-caesarean wound sepsis in mothers, showing a need to revise the routine materials used during surgery (Hinkson *et al,*. 2016). In multiparous mothers, development of wound sepsis after caesarean section has been shown to be associated with uterine wound dehiscence and this warrants further investigations to be conducted in affected mothers to improve on their prognosis (Bharatam *et al.*, 2015).

Current evidence has also shown that women who received both chlorhexidine-alcohol and iodine-alcohol for skin antisepsis at caesarean section compared to those who received one had a lower risk for the development of post-caesarean wound sepsis (Temming *et al.*, 2017). The same study also showed that obesity, smoking and presence of non-communicable diseases, obstetrician experience and skin incision type, were not major factors in patients who received prophylactic antibiotics within 60 minutes of caesarean section and prior to skin incision (Dlamini *et al.*, 2015). Application of chlorhexidine-alcohol skin antiseptics within 3 minutes to skin incision and closure of the subcutaneous layer when it was greater than two centimetres deep reduced the risk of post-caesarean wound sepsis (Temming *et al.*, 2017).

Caesarean section is often indicated following fetal distress, prolonged second stage labor, breech, and cephalo-pelvic disproportion. However, the development of sepsis means that patients would stay longer in the hospital than necessary probably as a result of severe hemorrhage during parturition (Mylonas & Friese, 2015). Mismanagement of sepsis of bacterial origin as a result of poor post-operative care has been shown to be associated with high maternal mortalities the prevalence of which is highest in developing countries, thus leading to the surviving sepsis campaign which advocates for improved management of patients (Acosta *et al.*, 2013). This is important since poor post-operative care would lead to increased maternal and neonatal morbidity (Oboro *et al.*, 2010) which would lead to decreased incidence of wound infections, thus reducing the risk posed by sepsis to post-operative in the community.

In Norway, low level of education of patients has been associated with a higher risk for caesarean sections wound sepsis as compared to the highly educated group (Tollåne *et al*, 2007). This has led to a lot of controversy as to whether caesarean sections wound sepsis are basically for the poor in developed countries. In addition a study by Cesaroni *et al.*, (2008) showed that women with a primary level of education had an over 20% risk for caesarean sections wound sepsis than those who had attended university.

Hygiene has also been associated with the ability to influence microbial colonization of the wound following caesarean sections thus affecting the immune status of the patient (Neu & Rushing, 2011). This shows that improved hygiene leads to improved patient outcomes. In addition, caesarean sections wound sepsis have been associated with socio-economic status that is to say, low in poor communities (employment), high in highly educated patients, being married and age (Faisal *et al*,. 2017). According to a recent study done in Rwanda patients who are referred from lower health units usually present in critical conditions and their prognosis are poorer compared to those who are not referred (Kalisa *et al*,. 2016).

2.3 Common bacterial pathogens in post-caesarean wound sepsis

The common bacterial pathogens causing sepsis in the hospital (Royal College of Obstericians & Gynaecologists, 2012) include *Streptococcus pyogenes, Staphylococcus aureus,* coliforms, *Streptococcus pneumonia, Clostridium septicum*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Morganella morganii.* In East Africa, the prevalent pathogens are *Staphylococcus aureus,* coliforms, *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli*, and *Enterobacter Spp* (Anguzu

& Olila, 2007; Sekirime & Lule, 2009) which shows an interplay of both Gram-negative and Gram-positive bacteria. In addition, Cordioli *et al.*, (2013) has shown that the major Gram-negative bacteria in post-caesarean sepsis are *Escherichia coli, Hemophilus influenza, Klebisiella spp., Enterobacter spp., Proteus spp., Pseudomonas spp., Serratia spp.* Furthermore, the major Gram-positive bacteria (Cordioli *et al.*, 2013) have been reported to include *Pneumococus, Streptococcus* groups A, B and D., *Enterococcus, Staphylococcus aureus, Listeria monocytogenes,* while the major anaerobic bacteria have been shown to be *Bacteriodes species, Clostridium perfringens, Fusobacterium species, Peptococcus* and *Peptostreptococcus.* These observations show that aerobic bacteria are a major concern in the development of post-caesarean wound sepsis.

2.4 Antibacterial drug Susceptibility Patterns of Bacterial Isolates

In the management of post-caesarean wound sepsis, early identification of the problem and constitution of the appropriate therapy is important in the improvement of patient's prognosis (Moores, 2013). However, with the increasing burden of antibiotic resistance, offering the right treatment is currently a challenge, especially in developing countries were laboratory costs are highly exaggerated (Ezeonwumelu *et al.*, 2016). This is important since bacterial culture is important in identifying major pathogens and making appropriate and effective diagnoses (Bonham, 2009; Cheesbrough, 2006). This is highly important since prophylactic treatment has been shown to improve on post-operative wound healing in a maternal population (Dlamini *et al.*, 2015).

Antibacterial sensitivity is important for effective management of post-caesarean wound sepsis of bacterial origin since treatment failures lead to a poor prognosis in affected patients (Anguzu., 2007). Under normal conditions, large numbers of the peripheral blood neutrophils enter sites of bacterial infection by first adhering to activated endothelial cells and then migrating along a gradient of chemotactic factors. In contrast, neutrophils from septic patients have increased expression of surface integrins which promote firm adhesion to endothelial cells. As a sequence, the neutrophils remain bound more tightly to the endothelial cells and fail to migrate appropriately into the site of bacterial infection (Jacobi, 2002).

At Mbarara Regional Referral Hospital(Uganda), antibacterial resistance has been shown to be highest among the penicillins and their derivatives (Bebell *et al.*, 2017), highlighting the need to identify the sensitivity profile in post-partum women after caesarean section. A study conducted at Mulago Hospital, in Central Uganda, has also shown that antimicrobial

resistance to various antimicrobial agents is a real threat (Kateete *et al.*, 2011). In Tanzania, general resistance to antimicrobial agents has also been found to be high with gentamicin being found to be the only effective antibacterial agent against the isolates (Dhar *et al.*, 2014). This implies that without an updated susceptibility profile in a given population, it would be challenging for clinicians to effectively manage sepsis in post-caesarean mothers (Royal College of Obstericians & Gynaecologists, 2012).

2.4.1 Methods for antimicrobial susceptibility testing

Due to the high burden of bacterial resistance to antimicrobials, there has been interest in drug susceptibility testing to ensure good treatment outcomes. Two main methods are used for susceptibility testing which are the disc diffusion and minimum inhibitory concentration (MIC) tests for aerobic bacteria (Tenover, 2009). Selection of the most appropriate antimicrobial agents to test and to report is a decision best made by each laboratory in consultation with the infectious diseases practitioners and the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the medical staff (Cavalieri, Rankin, Harbeck, & Sautter, 2005).

The disk diffusion method has several steps. Once isolated colonies are available from an organism that has been identified as a potential pathogen, it is necessary to proceed to perform the susceptibility test as follows: The colonies are selected first and then the inoculum suspension is prepared and standardized. Inoculation of the plate is then done. The antimicrobial disks are added and then the plates are incubated. The diameters of the zones inhibition are then measured and the results interpreted using the criteria by Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) (CLSI, 2017; Cavalieri *et al.*, 2005).

The other common method is the minimum inhibitory concentration test. The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system. The concentration in the laboratory is determined by incubating a known quantity of bacteria with specified dilutions of the antimicrobial agent. The results are interpreted as susceptible, intermediate, or resistant using the criteria for the Clinical Laboratories (CLSI, 2017). The MIC tests can be done by either broth or agar media, but broth microdilution is the most widely used method in clinical laboratories. MIC panels

that contain dilutions of one or multiple antimicrobial agents in a broth microdilution format are on market and are cleared before use by Food and Drug Authority.

2.4.2 Performing MIC vs disk diffusion tests

MIC tests are required for some organisms/antimicrobial combinations for which disk diffusion testing has proven to be unreliable (Cavalieri *et al.*, 2005; Tenover, 2009). These include:

i. *Streptococcus pneumoniae* which requires that MIC test be performed for penicillin when isolates show zones of inhibition <20 mm around oxacillin disks (a screening test for penicillin resistance), and also MIC tests for cefotaxime or ceftriaxone because breakpoints for disk diffusion testing have not been established for these agents.

ii. *Viridans streptococci* which also require determination of MICs when isolates are from normally sterile body sites.

iii. *Staphylococcus species* which require that MIC tests be performed to detect decreased susceptibility to vancomycin since this cannot be determined using the disk diffusion test. These specific methods are mainly recommended for use when the isolates are resistant to the common antibiotics. This was in one of the studies in Uganda with commendable outcomes (Najjuka *et al.*, 2016). Antimicrobial susceptibility pattern of isolated bacterial pathogens will be performed by Kirby Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute. We chose this method as it is the one recommended by the CLSI (CLSI, 2017).

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Study design

This was a cross-sectional study because the purpose of the study was to determine prevalence, identify factors, common bacterial pathogens from post-caesarean wounds and antibacterial susceptibility pattern at Hoima Regional Referral Hospital.

3.2 Study site and setting

The study was conducted in the postnatal ward at Hoima Regional Referral Hospital which is a public hospital. Hoima District has GPS coordinates 01 24N, 31 18E and is approximately 230 km by road from Kampala, which is the capital city of Uganda. The major tribe is Banyoro and the main religions are Islam and Christianity; and the majority of the population are cultivators and animal keepers.

Hoima Regional Referral Hospital is a well-established hospital and it offers both in-patient and out-patient services with a range of departments and clinics, including General Surgery, Obstetrics and Gynecology and Internal Medicine. The hospital is well equipped with a bed capacity of 400. The Obstetrics and Gynecology Department of Hoima Regional Referral Hospital has four specialists, one Resident doctor, five intern doctors and 13 midwives. The obstetrics and gynecology department has 110 beds.

According to the hospital records (HRRH semi-annual maternal report, 2017), Hoima Regional Referral Hospital performs approximately a minimum of 10 caesarean sections per day. These are done in two theatres that are shared by other surgical teams. The hospital had a range of 10-20 vaginal deliveries per day and also offers antenatal and postnatal services. The main laboratory of Hoima Regional Referral Hospital consists of the following sections: hematology and blood bank, chemistry, parasitology and microbiology. It was composed of 20 staff members and these include three specialists, two laboratory technologists, eight laboratory technicians, six laboratory assistants, and one laboratory attendant. The exudate swab samples were processed in the microbiology laboratory which was operated by one laboratory technologist, one laboratory technician, one laboratory assistant and one laboratory attendant. It was well equipped to carry out culture and sensitivity and other microbiological tests. Some of the equipment found in this laboratory were; autoclave, incubator, microscope, hot air oven, refrigerator, safety cabinet and gas cylinder. It also had enough stains which

were used in the processing of samples. These include crystal violet solution, Lugol's iodine, neutral red solution and 50% acetone alcohol.

3.3 Study population

The study population were mothers who have delivered by caesarean section at Hoima Regional Referral Hospital during the period of the study.

3.4 Selection criteria

3.4.1 Inclusion Criteria

All adult and emancipated minor mothers (on ward or re-admitted) who would have delivered by caesarean section at Hoima Regional Referral Hospital with or without post-caesarean wound sepsis.

3.4.2 Exclusion Criteria

Those done caesarean section from other health units and then referred to Hoima regional referral hospital were excluded due to limited access to their medical records. Mothers who would have had a re-exploration due to caesarean section complications other than suspected sepsis, as well as those who were in their early puerperium and those who reported after six weeks, were also excluded.

3.5 Sample size

The minimum sample size for this study was 271.

3.6 Sample size determination

Specific objective one: The prevalence of post caesarean wound sepsis in Uganda was found to be 22.2% (Hassan & Alegbeleye, 2018).

Using formula (Daniel, 1999);

$$n = \frac{(z_\alpha)^2 x\, px\, (1-p)}{e^2}$$

Where:

Z = Standard normal deviate at 95% level of confidence; z= 1.96

Z_α= z-statistic at α=1.96

p = prevalence of post-caesarean wound sepsis in Uganda, p=22.2%

e = level of precision (in proportion of one, if 5% e=0.05)

n = Desired sample size

$$n = \frac{(1.96)^2 x\ 0.222x\ (1 - 0.222)}{0.05^2} = 266$$

Objective two: The associated factors of post-caesarean wound sepsis; the sample size was determined according to modified Daniel's formula (Daniel, 1999);

$$n = \frac{(z_\alpha + z_\beta)^2 x \frac{1}{R}\ px\ (1 - p)}{e^2}$$

Where:

Z = Standard normal deviate at 95% level of confidence; z= 1.96

$\mathbf{Z_\alpha}$= z-statistic at α=1.96, $\mathbf{Z_\beta}$ = z-statistic at β=0.84

p = prevalence of post-caesarean wound sepsis in Uganda, p=22.2%

e = level of precision (in proportion of one, if 5% e=0.05)

n = Desired sample size

R = Odds ratio=2.0

$$n = \frac{(1.96 + 0.84)^2 x\ \frac{1}{2}\ x\ 0.222x\ (1 - 0.222)}{0.05^2} = 271$$

Objective three & four: The common bacterial cause of post-caesarean wound sepsis, *Pseudomonas aeruginosa*, 21% in Muhimbili University teaching hospital (Manyahi, 2012); Using formula; (Daniel, 1999)

$$n = \frac{z^2 pq}{d^2}$$

Where;

n = Desired sample size

z = Standard normal deviate at 95% level of confidence; z= 1.96

p = expected prevalence of post-caesarean wound sepsis, p=0.21

d = level of precision (in proportion of one, if 5% d=0.05)

$$n = \frac{(Z_\alpha)^2 x\, p(1-p)}{(d)^2}$$

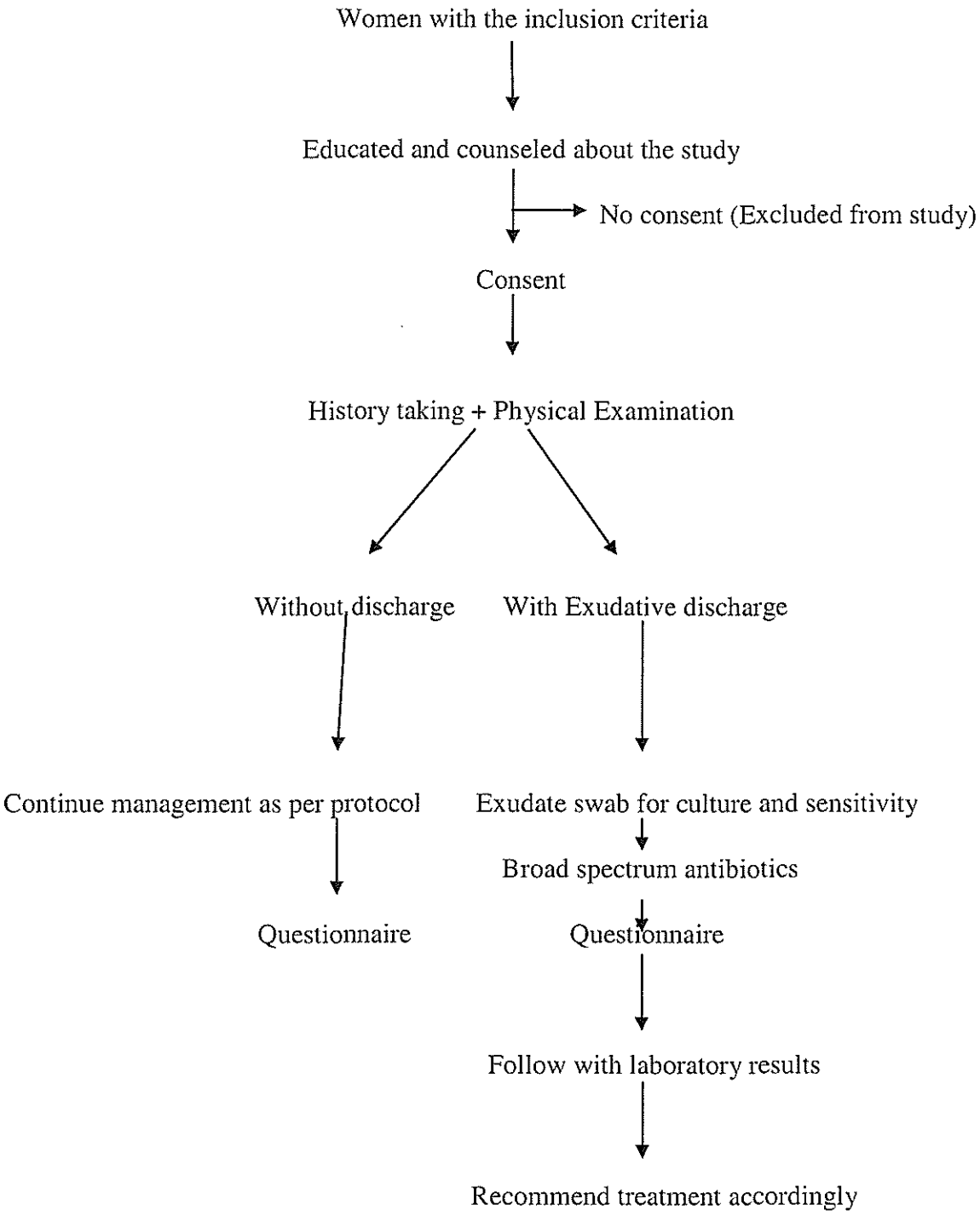
$$n = \frac{(1.96)^2\, x\, 0.21x\, (1-0.21)}{0.05^2} = 255$$

Therefore the overall minimum sample size for this study was 303 participants.

3.7 Sampling technique

Consecutive enrollment of participants who consent to participate in the study. This was carried out on a daily basis until required sample size.

Figure 2: Data collection Scheme



3.8 Data collection instruments

Structured investigator-administered pre-tested questionnaire was used for each participant to collect information on socio-demographic and known factors that may be related to the development of post-caesarean wound sepsis in each patient including obstetric factors, hospital factors and health factors.

A detailed history was elicited (English), translated where necessary for women who did not understand English; and physical examination was performed. Presence or absence of post-caesarean wound discharge (exudate) was noted. Swabs for mothers with discharge were taken and cultured in the laboratory according to standard clinical laboratory guidelines. Susceptibility testing was carried out according to Kirby Baur diffusion methods.

3.9 Sample collection and transportation

Patients with post-caesarean wound sepsis who met the inclusion criteria of the study were educated and counseled about the study and those who consented to participate, were recruited for the study. They were requested to allow history taking and physical examination and when a patient had post-caesarean wound discharge (Exudate), a sample was taken for microbiological analysis. Using sterile swab sticks, two samples from each participant were collected by gently rubbing the sterile swab sticks in the infected site (wound depth) using aseptic technique and immediately replaced inside the swab sticks case. The sterile swab stick was labeled with each participant's study number and transported to Microbiology laboratory immediately for processing, and in any delay, the sample was stored aerobically in the refrigerator at 4-8^{o}C.

3.10 Validity of data collection instruments

The data collection instruments was pretested in an independent laboratory (Kampala International University Teaching Hospital) to identify possible sources of errors that may arise during data collection. To establish the Content Validity Index, 15 respondents who were not part of the sample population were administered a questionnaire to measure the inter-respondent agreement. The agreement of more than 78% was a measure that the items of the questionnaire could provide a picture of factors associated with post-caesarean wound sepsis.

3.11 Reliability of data collection instruments

Data was obtained by a pre-determined questionnaire and by using the Cronbach's coefficient alpha of more than 0.8, the items of the questionnaire are checked for reproducible and consistent. The specimen was collected while ensuring sterile conditions so that reliability was ensured.

3.12 Sample processing and analysis

3.12.1 Isolation

The collected samples were inoculated on blood agar, chocolate agar, MacConkey agar and mannitol salt agar. They were then incubated both aerobically and anaerobically at 37^{0}C for 24-48hrs.

3.12.2 Direct Gram Microscopy

A direct smear was made for Gram stain; a drop of sterile normal saline was added at the center of a clean dried glass slide and the swab containing the sample rolled in the drop of normal saline spreading it on the glass slide in a circular motion to make a thin smear of the size of a fifty shilling coin. The smear was allowed to air-dry and then heat-fixed by passing it at least three times over a Bunsen flame. The slide was placed on the staining rack and flooded with crystal violet solution for 60 seconds, washed with clean water and covered with Lugol's Iodine (a mordant) and then allowed to act for a minute.

The slide was again washed in clean water and then decolorized with 50% acetone- alcohol under slow running tap water until a faint pink color was observed or no more color tend to flow from the smear. The process of decolorizing did not exceed 30 seconds. After decolorizing, the slide was washed in clean water and counterstained with neutral red solution. The slide was then washed in clean water; air-dried and observed under the microscope with x100 objective lens (oil immersion lens). Gram-positive bacteria was observed as blue or purple color and Gram-negative as red or pink color. Also, the morphology and shape of the bacteria was used to identify whether they are cocci, diplococcic, cocci in chains, clusters, and whether they are rods in appearance. Pus cells were also observed in the direct Gram-stained slide.

3.12.3 Identification of bacterial isolates

3.12.3.1 Cultural characteristics

The colony morphological characteristics of the bacterial isolates were observed as follows; color, margin, mucoid, texture, and hemolysis on blood agar medium, among others. This helped in determining the characteristics of the colonies of the bacteria on culture media such as Lactose or non-lactose fermenters on MacConkey agar and type of hemolysis (alpha, beta, and gamma hemolysis) on blood agar.

3.12.3.2 Biochemical tests

The isolates were identified using the biochemical tests that included catalase, optochin, bacitracin, coagulase, indole, citrate utilization, urea utilization, triple sugar iron agar fermentation, MR-VP test and oxidase as described below:

i. Catalase test

The Catalase Test was carried out to differentiate between *Streptococcus* and *Staphylococcus* species and this was done according to the method described by Cheesbrough, (2006), to determine the ability of the isolate to produce the enzyme, catalase. A drop of 3% hydrogen peroxide was added to a loop full of the test organisms. Presence of bubbles indicated catalase activity. *Streptococcus* species was catalase positive while *Staphylococcus species* was catalase negative.

ii. Indole test

The Indole Test was carried out according to the method described by Cheesbrough, (2006) to determine the ability of the isolate to degrade amino acid tryptophan and produce tryptophanase enzyme. A 1% tryptophan broth in a test tube was inoculated with 7 days isolate and incubated at 37°C for 48 hours. After 48 hours, 1 ml of chloroform was added to the broth. The test tube was shaken gently, and 2.1 ml of Kovac's reagent was added and again shaken gently. This was allowed to stand for 20 minutes. The formation of red coloration at the top layer, indicated a positive test, while a yellow coloration indicated negative result. *Escherichia coli* and *Proteus* are indole-positive.

iii. Urease test

The Urease test was carried out according to the method described by Cheesbrough, (2006) to determine the ability of the bacteria to hydrolyse urea and produce ammonia and carbon dioxide. The test organism were inoculated into urease broth and incubated at 30°C for 72 hours. Purplish pink coloration of the medium indicated a positive reaction for *Proteus* and negative for other enterobacteria like *Klebsiella* and *E. Coli*.

iv. Citrate utilization

This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and incubated for 24 to 72 hours. The development of deep-blue color after incubation was indicate a positive result (Cheesbrough, 2006). *Klebsiella* species are citrate-positive.

v. Triple sugar- iron test

Triple sugar iron test was carried out according to the method described by Cheesbrough, (2006); the test determined the ability of the organism to ferment the three sugar component of the medium: glucose, lactose and sucrose. The medium contains a pH indicator (phenol red) and a detection system (thiosulphate and ferrous sulphate) for hydrogen sulphide (H_2S). The medium was prepared as an agar slant. The test organism was inoculated by stabbing the medium using sterilized straight wire loop and the surface of the slope was also streaked with the test organism. The test was incubated at 37°C for 3 days. After incubation, gas production was determined by observing the cracking of the medium, and production of H_2S was observed by the blackening of the butt (bottom) of the medium. The triple-sugar iron-agar aided in identification of *Escherichia coli* which ferments all three sugars and produce acid, turning the media into yellow color. *Proteus* species produces H_2S which is indicated by black coloration of the media and fermentation at the butt of the tube.

vi. Methyl red – Voges – Proskauer test (MR-VP)

Methyl red – Voges – Proskauer test (MR-VP) was carried out according to the method described by Cheesbrough, (2006). It was used to determine the ability of the organisms to ferment glucose with production of acid. Five milliliters (5 ml) of MR-VP broth were inoculated with the test organism and incubated for 48 to 72 hours at 37°C. After incubation, 2 to 3 drops of methyl red test were added to 1ml of the broth. A red color signified a positive methyl red test, while yellow color signified a negative test. To what remained, five drops of 4% potassium hydroxide (KOH) were added followed by fifteen drops of 5% α –naphthol in ethanol. The development of red color within 1 hour indicates VP positive test while no color change indicated VP negative test. *Escherichia coli* is methyl red positive and voges-proskauer negative.

vii. Coagulase test

This test was carried out according to the method described by Cheesbrough, (2006). It was used to identify *Stapylococcus aureus* which produces the enzyme coagulase. The rapid slide test was done by placing a drop of distilled water on each end of slide. Then a colony of the test organism (previously checked by Gram-staining) was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspensions (no plasma was added to the second suspension), and mixed gently. Formation of clumps of the organisms within 10 seconds was indicative of a positive test while absence of these clumps was indicative of negative results.

For suspected *Staphylococcus aureus* isolates which turn negative for the rapid slide test, the test was done by emulsifying several isolated colonies of test organism in 1 ml of diluted rabbit plasma (1:5) dilution to give a milky suspension. The tubes were then incubated at 35°C in water bath for 4 hours. These were then examined at intervals of 1, 2 and 4 hours for clot formation by tilting the tube through 90°. If the test was still negative, the tube was left at room temperature overnight and examined again for *Staphylococcus aureus* that produced a delayed clot.

viii. Oxidase test

The test was used in identification of organisms which produce the enzyme cytochrome oxidase. A filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride was moistened with sterile distilled water. Using a glass rod, a colony of the test organism was smeared on the filter paper. The development of a blue-purple color within a 10 seconds was indicative of positive test while absence or formation of a blue-purple color after 10 seconds was considered negative (Cheesbrough, 2006). *Pseudomonas* species and *Neisseria* species are oxidase positive.

3.12.4 Susceptibility Pattern Determination (Kirby-Bauer disc diffusion technique)

The susceptibility pattern of the identified pathogens was determined by agar disc diffusion technique by Kirby-Bauer using Muller Hinton agar. The Muller Hinton agar was prepared according to the manufacturer's instructions under sterile conditions to avoid any contamination that may result. About 4-5 colonies of the organism were diluted in sterile peptone water and mixed and incubated for 4-5 minutes and its turbidity compared to a McFarland standard 0.5%.

A prepared Muller Hinton agar was dried in an incubator for at least 15 minutes and using a sterile glass rod or swab stick, the surface were smeared with the diluted organism of the peptone water, and using sterile forceps, different antibiotic containing discs were placed on the dried surface of the Muller Hinton agar containing the organism and then placed in the incubator at 37°C for 24 hours.

The diameter of a clear zone surrounding the antibiotic disc was measured in millimeters and compared to a standard antibiotic chart for measuring zones of inhibition. Zones of inhibition measured were recorded as susceptible (S), Intermediate (I), and Resistant (R) according to the standard chart (Cheesbrough, 2006; Dlamini *et al.*, 2015).

3.13 Quality control

Inclusion and exclusion criteria were strictly adhered to. A common pretested questionnaire which was edited prior to their use was used. The questionnaires was checked for completeness before collection to ensure valid data is obtained. Under the guide of two independent microbiologists from different laboratories characterization of the bacterial isolates was conducted to a maximum. Antibiotic discs selected for the study reflected the antibiotics easily accessible on the Ugandan market to achieve the susceptibility patterns. Samples were labelled according to the numerical code of the participants for easy identification and follow up. The Runyoro consent was back translated to English to see if it was similar.

3.14 Data analysis

Data on questionnaires was entered in Microsoft Excel version 2010, and then data from Excel was exported to IBM SPSS statistics version 23 as well as STATA 14.2 (Statacorp, USA Texas). Socio-demographic and clinical factors were summarized as means and medians, standard deviations and interquartile range (for continuous variables). Proportions, percentages and frequencies were used for categorical variables using STATA 14.2.

Objective one: Prevalence of post caesarean wound sepsis was summarized as frequencies and percentages and at 95% CI.

Objective two: The factors associated with post-caesarean wound sepsis were assessed using logistic regression. Binary logistic Regression was carried out using STATA version 14.2 to obtain Odds Ratio. For factors with p-value ≤ 0.2 at bivariate and those with biological plausibility were carried for multivariate analysis.

Objective three: Percentages were computed for each of the individual bacteria isolates.

Objective four: Percentages were computed for each of the individual bacteria isolates which are; Susceptible (S), Intermediate (I) and Resistant (R). Information was presented in bar graphs.

3.15 Ethical considerations

3.15.1 Informed consent and respect for participants

Voluntary recruitment was done and an informed consent was signed. Informed consent from participants was obtained after fully explaining the details of the study to them in English and local languages (copy attached at Appendices I and II). Emancipated minors required presence of their guardians to consent. Participants were not forced to enroll themselves if they did not want to. Participant were free to withdraw from the study at any time as they wish without coercion or compromise of care they are entitled to.

3.15.2 Risks and adverse events to study participants

Post-caesarean mothers may undergo pain during swabbing. However, the process of obtaining a swab was done gently and professionally to minimize risk of pain and minimize re-infection as much as possible. Additionally, culture and sensitivity tests are the recommended guidelines prior to antibiotic therapy to minimize the risk of antibiotic resistance.

3.15.3 Benefits of the research

The study did not provide direct benefits to the participants other than ensuring that routine care for wound sepsis was provided. The community benefited as through disseminate major of research findings on the most active antibiotics that should be prescribed to post- caesarean mothers at Hoima Regional Referral Hospital. These findings have significantly played a key role in controlling sepsis at the study site.

3.15.4 Privacy and Confidentiality

Identification of participants was done by means of numerical codes. Details of respondents was kept under lock and key for privacy and confidentiality purposes throughout the course of research. Respect of the respondents' rights and fair treatment were strictly adhered to thus minimizing harm and discomfort to them. There was no disclosure of participants' names to the public and all identities were removed from the results before publication.

3.15.5 Selection of Participants

Consecutive sampling method was used to select participants to ensure equal chance of being selected for the study. Eligibility criteria was strictly adhered to and sample size was

sufficient enough since an observed power of 80% was used in computation. No bias was given in terms of tribe, interest group, race or religion.

3.15.6 Incentives and Reimbursement

No monetary or any other form of incentives was offered to the participants but compensation and reimbursement of funds used by participants for the purpose of being in the study was offered where applicable.

3.15.7 Approval Procedure

Approval to carry out the study was acquired from the department of obstetrics and gynecology, the faculty and post graduate directorate and finally the Kampala International University Research Ethics Committee (KIU-REC). After approval by the KIU-REC, the study was registered with the Uganda National Council for Science and Technology (UNCST). The UNCST certificate was presented to the hospital administration of Hoima Regional Referral Hospital prior to data collection to acquire permission to proceed with the study at the study area.

3.15.8 Respect for community

The procedures involved in this study did not go against the local community beliefs, traditions and culture. Findings from the study were communicated to the head of Obstetrics and Gynecology department of Hoima Regional Referral Hospital as a formal feedback as well as office of District Health Officer, Hoima district so that the community can gain from it.

3.16 Dissemination of results

A copy of study findings/results obtained will be sent to the Post-graduate directorate records department, to the department of obstetrics and gynaecology KIU-TH and HRRH, as well as office of District Health Officer, Hoima district. This work will be published in journals.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of study participants attending Hoima Regional Referral Hospital

Table 1. The median age was 23 years for both referred and non-referred study participants, with 92.2%married among the non-referred participants. Majority of the non-referred study participants had no formal education (56%) compared to 24% among referred participants (p=0.001). Farming was the major occupation (79%) among the referred participants with 41% Anglicans were the majority among the non-referred participants while believers of Bishaka faith were the minority (<5%) in both groups.

Table 1: Socio-demographic characteristics of study participants attending Hoima Regional Referral Hospital

	Patient type		
Variable	Non-referred (n=205)	Referred (n=98)	p-value
Median age in years (IQR)	23(20-28)	23(19-28)	0.95
Marital status n (%)			
Married	189(92.2)	87(88.8)	0.56
Single	9(4.4)	8(8.2)	0.51
Divorced	5(2.4)	2(2.0)	0.95
Cohabiting	2(1.0)	1(1.0)	1.00
Education n (%)			
None	115(56.1)	23(23.5)	**0.001**
Primary	49(23.9)	52(53.1)	**0.004**
Secondary	26(12.7)	19(19.4)	0.51
Tertiary	15(7.3)	4(4.1)	0.075
Employment n (%)			
Farmer	119(58.1)	77(78.6)	0.25
Health worker	2(1.0)	2(2.0)	0.95
Saloon	28(13.7)	7(7.1)	0.51
Teacher	9(4.4)	3(3.1)	0.94
Others	47(22.9)	9(9.2)	0.44
Religion n (%)			
Catholic	68(33.2)	39(39.80)	0.75
Anglican	84(41.0)	32(32.7)	0.69
Muslim	15(7.32)	4(4.1)	0.88
SDA	12(5.9)	4(4.1)	0.93
Bishaka	7(3.4)	4(4.1)	0.97
Others	19(9.3)	15(15.3)	0.78
Median no. of times bathe a day (sd)	1.94(0.64)	1.12(1.20	**<0.001**

4.2 Clinical characteristics of study participants attending Hoima Regional Referred Hospital

Table 2. The median BMI among non-referred participants is 27 compared to 26 in referred participants (p=0.02). The prevalence of diabetes Mellitus, Hypertension, HIV and Cancer was low, i.e. <1 %,< 7 %,< 3% and <1% respectively.

Table 2: Clinical characteristics of study participants attending Hoima Referral Hospital.

	Patient type		
	Non-referred (n=205)	Referred (n=98)	p-value
Median BMI (IQR)	27(25.0-29.9)	25.5(23.56-28.57)	**0.017**
History of diabetes			
No	204(99.51)	98(100.0)	0.31
Yes	1(0.49)	0(0.0)	**<0.001**
History of hypertension			
No	202(98.5)	91(92.86)	**0.04**
Yes	3(1.46)	7(7.14)	**0.04**
HIV			
No	199(97.1)	96(98.0)	0.63
Yes	6(2.9)	2(2.0)	0.63
History of cancer			
No	204(99.5)	98(100.0)	0.31
Yes	1(0.49)	0(0.0)	-

4.3 Hospital and obstetric factors of women attending Hoima Regional Referral Hospital

Table 3. In our study, intern doctors mainly handled caesarean sections for both referred and non-referred mothers attending Hoima Regional Referred Hospital. Emergency caesarean section were the majority in this setting with the most preferred type of incision being midline. Among 61% of non-referred patients, there were no antibiotics given before surgery compared to 42% among referred patients. Premature rupture of membranes was more among referred patients (13%) compared to Premature rupture of membranes among non-referred patients (p=0.03). Participants with more than one previous caesarean section were more in non-referred 29% compared to 25% in referred patient (p = 0.42)

Table 3: Hospital and obstetric factors of women attending Hoima regional referral Hospital

Variable	Patient type		p-value
	Non-referred (n=205)	Referred (n=98)	
Cadre that performed the surgery n (%)			
Intern	204(99.5)	96(98.0)	0.32
Medical officer	1(0.49)	2(2.0)	0.06
Antibiotics given prior surgery			
No	124(60.5)	41(41.8)	**0.02**
Yes	81(39.5)	57(58.2)	**0.02**
Median length of labor in hours (IQR)	9(6-13)	10(6-18)	0.07
Median parity (IQR)	2(1-3)	2(1-4)	0.30
Type of incision			
Transverse	86(42.0)	34(34.7)	0.16
Midline	119(58.1)	64(65.3)	0.23
Median no. of vaginal examination (IQR)	3(2-4)	2(1-5)	0.057
Type of c/section n (%)			
Emergency	190(92.7)	94(95.9)	0.24
Elective	15(7.3)	4(4.1)	0.24
Techniques of CS n (%)			
Classical	5(2.44)	6(6.12)	0.17
LTCS	200(97.56)	92(93.9)	0.17
Premature rapture of membrane			
No	193(95.1)	85(86.7)	**0.03**
Yes	10(4.9)	13(13.3)	**0.03**
Previous CS			
1	146(71.22)	74(75.5)	0.42
>1	59(28.8)	24(24.5)	0.42

4.4 Prevalence of post caesarean wound sepsis of women attending Hoima Regional Referral Hospital, western Uganda

Table 4. In the study comprising 303 participants, 16.8% were diagnosed with post-caesarean wound sepsis with non-referred and referred participants having the condition of 11.7% and 27.6% respectively

Table 4: Prevalence of post-caesarean wound sepsis of women attending Hoima Regional Referred Hospital, western Uganda

		Patient type				
Overall prevalence (n=303)		Non-referred (n=205)		Referred (n=98)		p-value
Frequency	%(95%CI)	Frequency	%(95%CI)	Frequency	%(95%CI)	
51	16.8(13.0-21.5)	24	11.7(7.9-16.9)	27	27.6(19.4-37.4)	**0.002**[a]

[a]P-value between prevalence of post-caesarean wound sepsis among referred and non-referred patients

4.5 Bivariate and multivariate logistic regression analysis of post-caesarean wound sepsis on associated socio-demographic factors among non-referred patients attending Hoima Regional Referral Hospital

Table 5. In the study single mothers were four times more likely to develop post-caesarean wound sepsis compared to married mothers (cOR=4.2, 95% CI 1.0- 18.2). Mothers who completed primary education were six times more likely to develop post-caesarean wound sepsis compared to those who didn't attain formal education (cOR=6.2, 95%CI 1.8-21.4). Mothers who completed secondary level were ten times more likely to develop post-caesarean wound sepsis than those who didn't attain formal education (cOR=10.2, 95% CI 2.7-38.3). Mothers who completed tertiary education were ten times more likely to develop post-caesarean wound sepsis compared to those who didn't attain formal education (cOR= 10.1, 95% CI 2.2-46.1). Anglican mothers were 60% times less likely to develop post-caesarean wound sepsis than Catholics (cOR= 0.4, 95% CI 0.1-0.2).

On adjusted analysis: Non-referred mothers who completed primary education were 5.5 times more likely to develop post-caesarean wound sepsis compared to those who never attained formal education (aOR=5.5, 95%CI 1.5-20.2). Non-referred mothers who completed secondary level were 11.6 times more likely to develop post-caesarean wound sepsis compared to those who never attained formal education (aOR=11.6, 95%CI 2.6-51.5). Non-referred mothers who completed tertiary level were 8.7 times more likely to develop post-caesarean wound sepsis compared to those who never attained formal education (aOR=8.7, 95%CI 1.8-43.1).

Table 5: Bivariate logistic regression analysis of post-caesarean wound sepsis on associated socio-demographic factors among non-referred patients attending Hoima Regional Referral Hospital

Variable n (%)	No sepsis (n=181)	Sepsis (n=24)	Crude analysis		Adjusted analysis	
			cOR(95%CI)	p-value	aOR (95%CI)	p-value
Age in years n (%)						
18-23	42(89.4)	5(10.6)	1.00			
24-28	43(86.0)	7(14.0)	1.37(0.40-4.65)	0.62		
>28	96(88.9)	12(11.1)	1.10(0.35-3.17)	0.93		
Marital status						
Married	169(89.4)	20(10.6)	1.00	-		
Single	6(66.7)	3(33.3)	4.23(1.00-18.22	**0.05**		
Divorced	4(80.0)	1(20.0)	2.11(0.22-19.8)	0.51		
Cohabiting	2(100.0)	0(0.0)	-			
Education						
None	111(96.5)	4(3.5)	1.00		1.00	
Primary	40(81.6)	9(18.4)	6.24(1.82-21.40)	**0.004**	5.50(1.50-20.17)	**0.01**
Secondary	19(73.1)	7(26.9)	10.22(2.73-38.32)	**0.001**	11.6(2.62-5.45)	**0.001**
Tertiary	11(73.3)	4(26.7)	10.09(2.21-46.05)	**0.003**	8.73(1.77-43.15)	**0.008**
Employment						
Farmer	107(89.9)	12(10.1)	1.00			
Health worker	2(100.0)	0(0.0)	-			
Saloon	25(89.3)	3(10.7)	1.07(0.28-4.08)	0.92		
Teacher	7(77.8)	2(22.2)	2.55(0.47-13.68)	0.28		
Others	40(85.1)	7(14.9)	1.56(0.57-4.24)	0.38		
Religion						
Catholic	56(82.3)	12(17.7)	1.0			
Anglican	78(92.9)	6(7.1)	0.36(0.13-1.0)	**0.05**		
Muslim	14(93.3)	1(6.7)	0.33(0.04-2.78)	0.31		
SDA	10(83.3)	2(16.7)	0.93(0.18-4.82)	0.93		
Bishaka	6(85.7)	1(14.3)	0.78(0.09-7.07)	0.82		
Other	17(89.5)	2(10.5)	0.55(0.11-2.70)	0.46		
Number of times bathed per day n (%)						
≤2	165(88.2)	22(11.8)	1.00			
>2	16(88.9)	2(11.1)	0.94(0.20-4.35)	0.69		
BMI						
Normal (18.5-25)	46(90.2)	5(9.8)	1.00			
underweight<18.5	0(0.00)	1(100.0)	-			
Overweight >25	134(88.2)	18(11.8)	1.24(0.43-3.52)	0.69		

4.6 Bivariate and Multivariate logistic regression analysis of post-caesarean wound sepsis on associated clinical, hospital and obstetric factors among non-referred patients attending Hoima Regional Referral Hospital

Table 6. Mothers who are HIV positive were 8.5 times more likely to develop post-caesarean wound sepsis than those who are HIV negative (cOR=8.5, 95%CI 1.6-44.7). Mothers who had prior caesarean section were 2.9 time more likely to develop post-caesarean wound sepsis than those without prior caesarean section (cOR=2.9, 95% CI 1.2-6.8).

On adjusted analysis: Non-referred mothers who were HIV positive were 6.4 times more likely to develop post-caesarean wound sepsis compared to those who were HIV negative (aOR=6.4, 95%CI 1.1-38.6). Non-referred mothers who experienced more than 4 vaginal examinations were four times more likely to develop post-caesarean wound sepsis compared to those done four times or less (aOR=4.0, 95% CI 1.1-14.3). Mothers with prior caesarean section were 3.5 time more likely to develop post-caesarean wound sepsis compared to those without (aOR=3.5, 95%CI 1.3-9.5)

Table 6: Bivariate and Multivariate logistic regression analysis of post-caesarean sepsis on associated clinical, hospital and obstetric factors among non-referred patients attending Hoima Regional Referred Hospital.

Variable n (%)	No sepsis (n=181)	Sepsis (n=24)	Crude analysis		Adjusted analysis	
			cOR (95%CI)	p-value	aOR(95%CI)	p-value
History of hypertension						
No	179(88.6)	23(11.4)	1.00			
Yes	2(66.7)	1(33.3)	3.89(0.34-44.62)	0.28		
HIV						
Negative	178(89.4)	21(10.6)	1.00		1.00	
Positive	3(50.0)	3(50.0)	8.48(1.61-44.71)	**0.012**	6.35(1.05-38.62)	**0.045**
Antibiotics given prior surgery						
No	111(88.0)	14(11.2)	1.00			
Yes	70(87.5)	10(12.50)	1.13(0.48-2.69)	0.78		
Length of labor in hours						
<12	126(86.3)	20(13.70)	1.00			
>12	49(92.5)	4(7.6)	0.51(0.17-1.58)	0.25		
Parity						
Primi-para 1	62(87.3)	9(12.7)	1.00			
Multipara (2-4)	10(89.8)	12(10.2)	0.78(0.31-1.96)	0.60		
Grand Multipara >4	13(81.3)	3(18.8)	1.59(0.38-6.69)	0.53		
Type of incision						
Transverse	77(89.5)	9(10.5)	1.00			
Midline	104(87.4)	15(12.6)	2.60(0.51-2.97)	0.64		
Times vaginal examination						
≤4	158(89.3)	19(10.5)	1.00		1.00	
>4	16(76.2)	5(23.8)	2.60(0.86-7.90)	0.092	4.00(1.10-14.28)	**0.035**
Types of CS						
Emergency	168(88.4)	22(11.6)	1.00			
Elective	13(86.7)	2(13.3)	1.17(0.25-5.56)	0.84		
PROM						
No	170(88.1)	23(11.9)	1.00			
Yes	9(90.0)	1(10.0)	0.82(0.10-6.78)	0.86		
Prior CS						
No	134(91.8)	12(8.2)	1.00		1.00	
Yes	47(79.7)	12(20.3)	2.85(1.20-6.78)	**0.018**	3.46(1.26-9.46)	**0.016**
Use of antiseptic						
No	34(82.9)	7(17.1)	1.00		1.00	
Yes	147(89.6)	17(10.4)	0.56(0.22-1.46)	0.24	0.53(0.16-1.83)	0.32

4.7 Bivariate and multivariate logistic regression analysis of post-caesarean wound sepsis on associated socio-demographic factors among referred patients attending Hoima Regional Referral Hospital

Table 7. Mothers who bathed more than two time were seven times more likely to develop post-caesarean wound sepsis compared to those who bathed two time or less (aOR = 7.0 ,95% CI 1.6-30.2). Referred Bishaka faith mothers were 6.8 times more likely to develop post-caesarean sepsis compared to catholics though it was statistically not significant (cOR=0.6, 95%CI 0.6-71.7). Referred mothers who bathed more than two times were 3.3 times more likely to develop post-caesarean wound sepsis compared to those who bathed two times or less though it was statistically not significant (cOR=3.5, 95%CI 0.9-11.2). Referred mothers who were underweight were 1.3 times more likely to develop post-caesarean wound sepsis compared to those with normal BMI though it was statistically not significant (cOR=1.3, 95% CI 0.5-3.4)

Table 7: Bivariate and multivariate logistic regression analysis of post-caesarean wound sepsis on associated socio-demographic factors among referred patients attending Hoima Regional Referral Hospital

Variable n (%)	No sepsis (n=71)	Sepsis (n=27)	Crude analysis		Adjusted analysis	
			cOR(95%CI)	p-value	aOR (95%CI)	p-value
Age in years						
18-23	16(76.2)	5(23.8)	1.00			
24-28	20(80.0)	5(20.0)	0.8(0.20-3.25)	0.76		
>29	35(67.3)	17(32.7)	1.55(0.49-4.95)	0.46		
Marital status						
Married	62(71.3)	25(28.7)	1.00			
Single	6(75.0)	2(25.0)	0.83(0.20-3.25)	0.76		
Divorced	2(100.0)	0(0.00)	-			
Cohabiting	1(100.0)	0(0.0)	-			
Education						
None	18(78.3)	5(21.7)	1.00			
Primary	38(73.1)	14(26.9)	1.33(0.41-4.25)	0.64		
Secondary	12(63.2)	7(36.8)	2.1(0.54-8.19)	0.29		
Tertiary	3(75.0)	1(25.0)	1.1(0.10-14.19)	0.89		
Employment						
Farmer	54(70.1)	23(29.9)	1.00			
Health worker	2(100.0)	0(0.0)	-			
Saloon	6(85.7)	1(14.3)	0.39(0.04-3.44)	0.40		
Teacher	2(66.7)	1(33.3)	1.17(0.10-13.60)	0.90		
Others	7(77.8)	2(22.2)	0.68(0.13-3.48)	0.63		
Religion						
Catholic	27(69.2)	12(30.8)	1.00			
Anglican	25(78.1)	7(21.9)	0.63(0.21-1.85)	0.40		
Muslim	3(75)	1(25)	0.75(0.07-7.79)	0.81		
SDA	3(75)	1(25)	0.75(0.07-7.97)	0.81		
Bishaka	1(25)	3(75)	6.75(0.64-71.71)	0.11		
Others	12(80.0)	3(20)	0.56(0.13-2.37)	0.43		
Number of time bathed per day						
≤2	65(76.5)	20(23.5)	1.00		1.00	
>2	6(50.0)	6(50.0)	3.25(0.94-11.20)	0.062	6.95(1.60-30.18)	**0.01**
BMI						
Normal	29(76.3)	9(23.7)	1.00	-	-	
<18.5	1(100.0)	0(0.0)	1.34	0.52-3.42	0.54	
>25	41(70.7)	17(29.3)				

4.8 Bivariate and Multivariate logistic regression analysis of post-caesarean wound sepsis on associated clinical, hospital and obstetric factors among referred patients attending Hoima Regional Referral Hospital

Table 8. Mothers who experienced more than four vaginal examinations were 3.8 times more likely to develop post-caesarean wound sepsis compared to those in whom four and less vaginal examinations were done (cOR=3.8, 95% CI 1.5-9.8). Referred mothers who were HIV positive were 2.7 times more likely to develop post-caesarean wound sepsis compared to those who are HIV negative although there was no statistical significance (cOR=2.7, 95%CI 0.2-44.5). Referred mothers who were hypertensive were 2.1 times more likely to develop post-caesarean wound sepsis compared to those without hypertension though there was no statistical significance (cOR=2.1, 95% CI 0.4-10.0). Referred mothers who were given antibiotics before surgery were 2.1 times more likely to develop post-caesarean wound sepsis compared to those who never received antibiotic though there was no statistical significance (cOR=2.1, 95%CI 0.8-5.3).

On adjusted analysis: Referred mothers who experienced more than four vaginal examinations were 6 times more likely to develop post-caesarean wound sepsis compared to those in whom four and less vaginal examinations were done (aOR = 6.0 95%CI 1.9-18.9).

Table 8: Crude and adjusted logistic regression analysis of post-caesarean sepsis on clinical, hospital and obstetric factors among referred patients attending Hoima Regional Referral Hospital

			Crude analysis		Adjusted analysis	
Variable n (%)	No sepsis (n=71)	Sepsis (n=27)	cOR (95%CI)	p-value	aOR(95%CI)	p-value
History of hypertension						
No	67(73.6)	24(27.1)	1.00			
Yes	3(57.1)	3(42.9)	2.09(0.44-10.04)	0.49		
HIV						
Negative	70(72.9)	26(27.1)	1.00		1.00	
Positive	1(50.0)	1(50.0)	2.69(0.16-44.64)	0.49	0.54(0.02-22.68)	0.69
Antibiotics given before surgery						
No	33(80.5)	8(19.5)	1.00			
Yes	38(66.7)	19(33.3)	2.06(0.80-5.33)	0.14		
Length of labor in hours						
≤12	44(75.9)	14(24.1)	1.00	-		
>12	27(67.5)	13(32.5)	1.51(0.62-3.70)	0.36		
Parity						
Primi-para	23(63.9)	13(36.1)	1.00	-		
Multipara	32(74.4)	11(25.6)	0.61(0.23-1.60)	0.31		
Grad Multipara	16(84.2)	3(15.8)	0.33(0.08-1.36)	0.13		
Type of incision						
Transverse	26(76.5)	8(23.5)	1.00			
Midline	45(70.3)	19(29.7)	1.37(0.51-3.57)	0.52		
No. of vaginal examination made						
≤4	57(80.3)	14(19.7)	1.00		1.00	
>4	14(51.9)	13(48.1)	3.78(1.46-9.82)	**0.006**	6.04(1.93-18.96)	**0.002**
Type of CS						
Emergency	68(72.3)	26(27.7)	1.00			
Elective	3(75.0)	1(25.0)	0.87(0.09-8.76)	0.91		
PROM						
No	62(72.9)	23(27.1)	1.00			
Yes	9(69.2)	4(30.8)	1.20(0.34-4.27)	0.78		
Prior CS						
No	50(67.6)	24(32.4)	1.00		1.00	
Yes	21(87.50)	3(12.50)	0.30(0.08-1.10)	0.07	0.26(0.06-1.09)	0.07
Use of antiseptic						
No	12(46.2)	14(53.8)	1.00		1.00	
Yes	59(81.9)	13(18.1)	0.19(0.07-0.50)	**0.001**	0.20(0.05-0.70)	**0.012**

4.9 Common bacterial pathogens that were isolated among study participants attending Hoima Regional Referral Hospital

Table 9. The most common bacterial isolates were *Staphylococcus aureus* 28.8%, followed by E. *coli*, 21%. The least was *Proteus mirabilis.*

Table 9: Common bacterial isolates from post-caesarean septic wounds from Mothers in Hoima Regional Referral Hospital, Uganda.

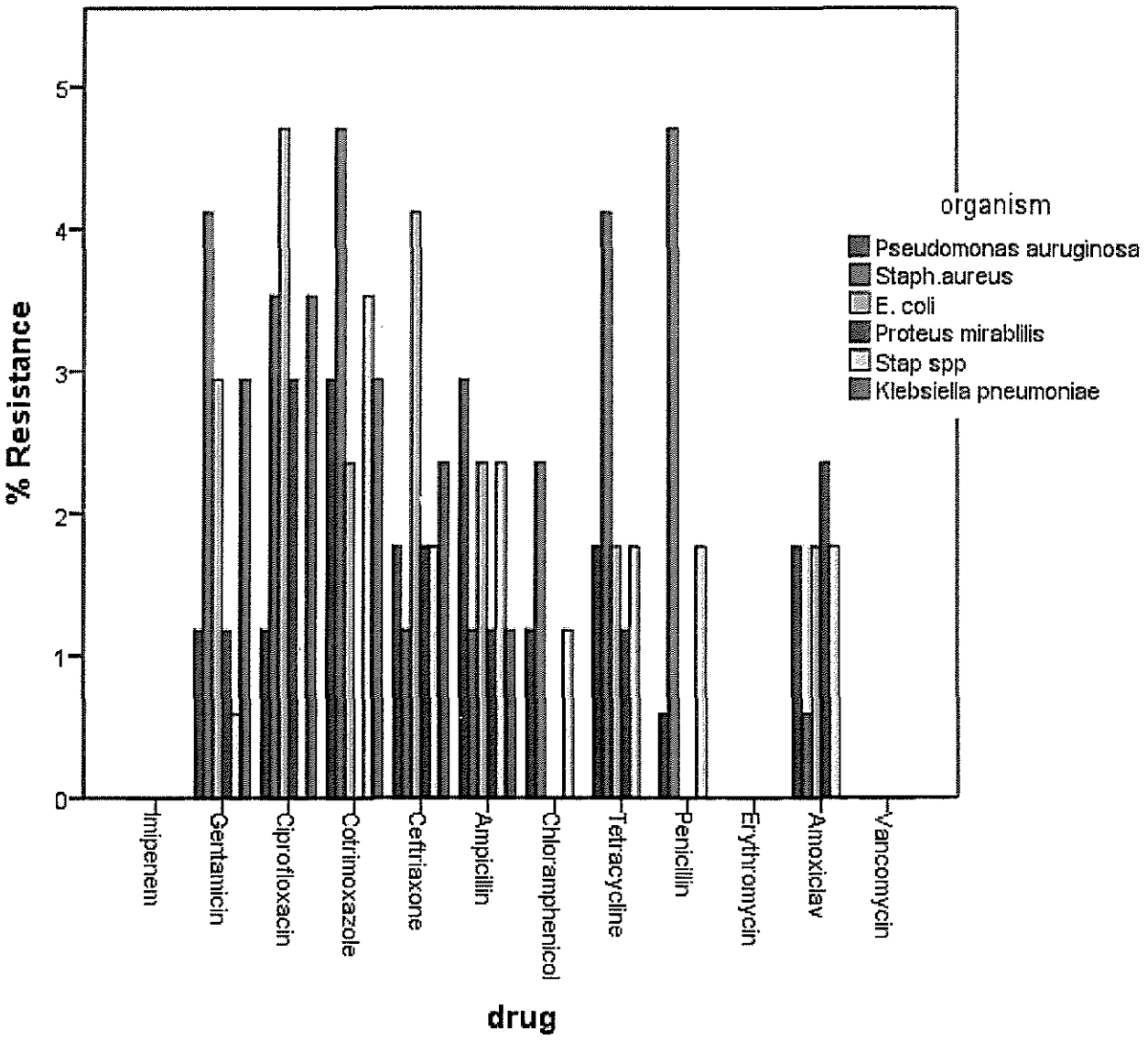
Pathogen	Frequency	Percent	95%CI
Pseudomonas aeruginosa	43	15.3	11.5-20.0
Staphylococcus aureus	81	28.8	23.8-34.4
E. *coli*†	59	21.0	16.6-26.2
Proteus mirabilis	24	8.5	5.8-12.5
Staphylococcus spp	35	12.5	9.1-16.9
Klebsiella pneumoniae†	39	13.9	10.3-18.5

†=coliforms

4.10. Drug susceptibility pattern of bacterial isolates from septic wounds of mothers in Hoima Regional Referral Hospital, Uganda

Figure 3: Ciprofloxacin, gentamicin, ceftriaxone,penicillin and cotrimoxazole were the most resisted drugs by *Staphylococcus aureus*, *E. coli* and *Klebsiella Pneumoniae*.

Figure 3. Resistance pattern of bacterial isolates against major antibiotics among mothers attending post-natal ward Hoima Regional Referral Hospital, Western Uganda

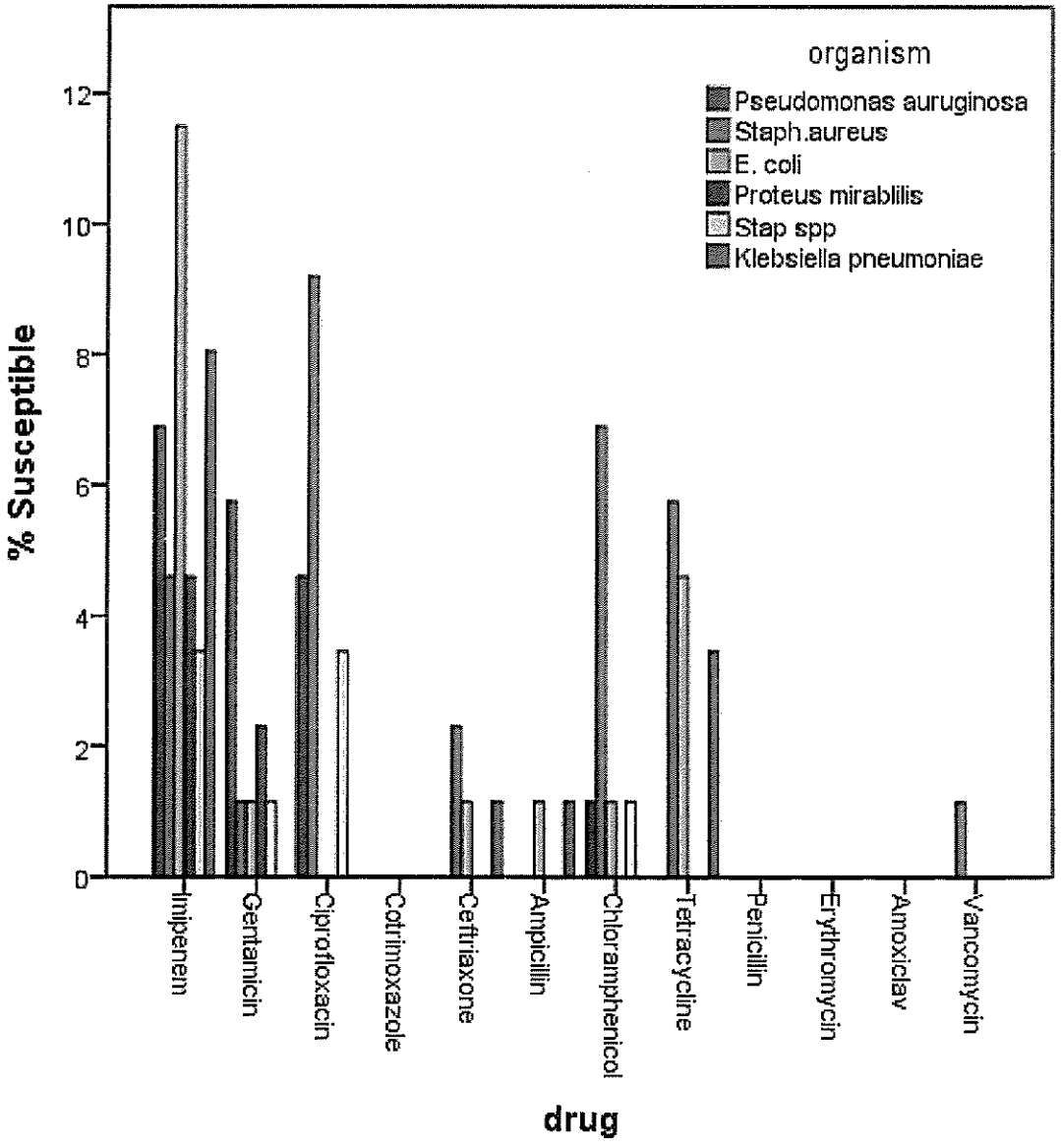


4.11. Drug susceptibility pattern of bacterial isolates from septic wounds of mothers in Hoima Regional Referral Hospital, Uganda.

Figure 4: Imipenem was the most active drug on all organisms especially on *E. coli, klebsiella pneumonae* and *Pseudomonas auruginosa.*

Coliforms showed high susceptibility to imipenem followed by *Staphylocccocus aureus* to ciprofloxacin.

Figure 4: Susceptibility pattern of bacterial isolates against major antibiotics among mothers attending post-natal ward Hoima Regional Referral Hospital, Western Uganda



CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

5.1 DISCUSSION

5.1.1 Prevalence of post caesarean wound sepsis among mothers attending post-natal ward at Hoima Regional Referral Hospital, Western Uganda

In our study the overall prevalence of post-caesarean wound sepsis was 16.8%. This was much higher than the reported Sub-saharan Africa prevalence of 7.3% by Chu *et al.*, (2012). The discrepancy could be due to small area of coverage within the population by the researcher as he only considered Burundi, DRC and Sierra Leone and generalized it to all sub-saharan Africa. Though Chu *et al.*, had a bigger sample size compared to this study, this study considered only one referral hospital. In addition, the current prevalence is also lower than that of 22.2% done in Kenyatta National Hospital. (Hassan & Alegbeleye, 2018). This may be due to fact that KNH is the biggest national referral Hospital in East Africa handling more cases than HRRH.

Our study findings show that post-caesarean wound sepsis was higher among referred patients than non-referred patients. This result could be due to complications such as obstructed labour that was higher among the referred patients (48%, 47/98) and this may be attributed to the multiple vaginal examinations before caesarean section and delayed ambulation after surgery. Our findings agree with those by Sai *et al* where 70% of the cases in Government Hospital, Tirupati, Chitoor district, India were due to obstructed labor (Sai *et al.*, 2016).

5.1.2 Risk factors of post caesarean wound sepsis among mothers attending Hoima Regional Referral Hospital, Western Uganda

High level of education (primary and post primary) was a significant predictor of post-caesarean wound sepsis among non-referred patients. This is contrary to the findings by Paschal *et al* from Northern Ghana where mothers with higher level education were less likely to experience post caesarean sepsis compared to those with primary and lower education (Apanga *et al.*, 2018). Our findings could be explained by non-compliance of educated mothers towards health education offered by health workers; in our setting there is

the "we know it all" among the educated mothers (especially secondary school education) as compared to mothers with no formal education. Also it may be due to increased maternal requests for caesarean sections which is a risk factor for post-caesarean wound sepsis among educated mothers (Abebe *et al.*, 2015). However, our findings are consistent with those obtained by Rajab *et al* in South Western Iran where mothers that had attained high school education were 1.4 times likely to have post-caesarean wound sepsis than those with lower school education (Rajabi *et al.*, 2015), and this suggests higher risk of post-caesarean wound sepsis among this group.

Behaviourally, mothers (non-referred) who reported having over 2 baths per day were more likely to experience post-caesarean wound sepsis compared to their counterparts who reported to have two or less number baths per day. This is against biological plausibility and it is possible that baths taken may be unhygienic as our participants were culturally oriented who incorporate herbs in their baths and this increases chances of microbial contamination of the wound post-caesarean section (Neu & Rushing, 2011). Additionally, the study participants were from low income background and possibility of having no recommended bathing soap is likely. There is need to sensitise such mothers against use of herbal concoctions in their baths.

Mothers who experienced more than four vaginal examinations had increased risk of post caesarean sepsis compared to mothers who experienced less than 4 vaginal examinations. Our findings are consistent with findings by Hassan *et al* obtained among mothers attending Port Harcourt Teaching Hospital, Southern Nigeria (Hassan & Alegbeleye, 2018). The similarity is the highest delivery rate between the two hospitals; Hoima Regional referral Hospital and Port Harcourt teaching Hospital. Frequent vaginal examinations are likely to introduce microbial pathogens during examination into uterus through the vagina.

In our study, mothers who were HIV positive were more likely to develop post-caesarean wound sepsis compared to HIV sero-negative mothers. Our findings are similar to those obtained by Marsel in Tygerberg Hospital, South Africa where HIV ART naïve patients were about 6 times more likely to experience post-caesarean sepsis (Coetzer, 2017). This can be explained by immunosuppression associated with HIV positivity that facilitate bacterial infection in such mothers.

In our study, mothers who had a prior caesarean section were more likely to have post--caesarean wound sepsis compared to those with no prior caesarean section. The explanation could be explained by several reasons: There are high chances of prolonged caesarean section for mothers with prior Caesarean section due to fibrosis and adhesion. Also, prolonged CS is likely to result into severe bleeding leading to anemia, delayed or no ambulation that have a significant role on risk of Post-caesarean wound sepsis.

We found out that antiseptic use (Savlon) was protective against post--caesarean wound sepsis among referred mothers and this is so because most of these mothers are from refugee camps (Kyangwali refugee resettlement) where they are supported with supplies like antiseptics. Our findings are consistent with those found by Kawakita and Landy in their review paper where use of Chlorhexidine (similar ingredient for savlon) as antiseptic was protective against post-caesarean wound sepsis (Kawakita, 2017).

We found that single mothers were more likely to have post-caesarean wound sepsis compared to the married mothers. This is possible because such mothers have got reduced care and support as compared to married ones.

Mothers of Anglican Faith were 64% .less likely to have post-caesarean wound sepsis compared to Catholics. In Anglican settings, there are formed groups such as Mother's Union which hold regular discussions /guidance about maternal health and hygiene among other activities. This has a bearing on reducing post-caesarean sepsis. Conversely catholic faith is liberal about alcohol consumption and this may have an implication on the risk of post-caesarean wound sepsis among the catholic mothers.

5.1.3 Common bacterial isolates

The study showed that the most common organisms were *Staphylococcus aureus* followed by *E. coli*. Findings in the current study are in agreement with findings in central Uganda in which *Staphylococcus aureus* and coliforms have been shown to be common pathogens in post-caesarean wound sepsis (Anguzu, 2007). In addition, in Zanzibar, *Staphylococcus aureus* and *E. coli* were found to be common pathogens in sepsis (Omar, 2010), showing similarities in the common pathogens of Tanzania and Uganda, this may be attributed to the fact that staphylococcus is a normal skin flora, and may have contaminated the wound during caesarean section since most of the work was handled by intern doctors who possibly had poor surgical techniques .In addition, a study done by Matinyi *et al.*,(2018) revealed that

staphylococcus was the most common organism isolated in hospital environment especially the door handles(100%) of Mbale Regional Referral Hospital (Matinyi *et al.*, 2018). However findings are different from the study done in Muhimbili teaching Hospital (Manyahi, 2012), where the commonest organism isolated was *Pseudomonas aeruginosa*, this may be due to the difference in the standard of Hygiene (poor aseptic technique) as evidenced in this Hospital, since one nurse was responsible for cleaning and dressing 15 post-operative wounds hence putting patients at risk of cross infection.

5.1.4 Resistance pattern of bacterial isolates

In our study, coliforms showed high susceptibility to Imipenem followed by *Staphylococcus aureus* to Ciprofloxacin and this is comparable to a study done in Mbale Regional Referral Hospital (Matinyi *et al.*, 2018). Furthermore, the study also showed that *Staphylococcus aureus* and other coliforms were resistant to Ciprofloxacin, Ceftriaxone, gentamycin and Cotrimoxazole. This may be attributed to the fact that these drugs were the most erroneously prescribed medications among the study population in Hoima Regional Referral Hospital. And hence these drugs are questionable as they are still commonly prescribed and widely used since they are more affordable than other antibiotics. The study also showed that *Staphylococcus aureus* was also resistant to Tetracycline and Penicillin which was in agreement with a study conducted by Bebell *et al* (2017). That showed over 80% antibacterial resistance to Penicillins and its derivatives in postpartum mothers of Uganda. In addition, a study done in Mulago National Referral Hospital revealed a development of *Staphylococcus aureus* methicillin resistance in post-caesarean wound sepsis (Kateete *et al.*, 2011). However, a study conducted in Tanzania reported a high resistance of *E.coli* to Gentamycin (Dhar *et al.*, 2014).

Strengths and Weakness of the study

It is the first documented study done at HRRH to report post-caesarean wound sepsis and associated bacterial organisms. Additionally, people who were re-admitted upon exudate were swabbed, so we minimized loss of follow-up and under reporting of Post-caesarean wound sepsis.

In our study, we did not swab the theater surfaces and aerial space to correlate environmental contamination and post-caesarean wound. We were unable to characterize the bacterial isolates at molecular level due to limited budget.

5.2 CONCLUSIONS

1. The prevalence of post-caesarean wound sepsis at Hoima Regional Referral Hospital is 16.8%.
2. The major significant risk factors of post-caesarean wound sepsis were being educated, history of previous caesarean section, hygiene, obstructed labor, HIV seropositivity and multiple vaginal examinations prior to surgery.
3. The most common bacterial pathogens were *Staphylococcus aureus* and the least was *Proteus mirabilis.*
4. Resistance was highest for coliforms and *Staphylococcus aureus* against, ciprofloxacin, gentamycin, penicillin, ceftriaxone and cotrimoxazole.

5.3 RECOMMENDATIONS

1. The hospital management should alert the health workers about the high prevalence of post-caesarean sepsis at this hospital to devise means of controlling it.
2. Enhanced awareness to the health workers and the patients on the major risk factors of caesarean wound sepsis and attending to them during management.
3. Health workers should enhance hygiene amongst mothers before, during and after caesarean section.
4. Educating Health workers on rational use of antibiotics especially with antibiotics that target *Staphylococcus aureus.*
5. I recommend a study that will involve swabbing theater and aerial space to correlate environmental contamination and post-caesarean wound sepsis.

5.4 LIMITATIONS

The study didn't follow up the mothers for a long period since this was a prevalence study in comparison to a cohort or case-control study which would help identify key variations in the risk factors. In addition, study didn't establish the resistance genes of staphylococcus.

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Appendix I: INFORMED CONSENT

KAMPALA INTERNATIONAL UNIVERSITY (KIU) WESTERN CAMPUS (WC)

RESEARCH ETHICS COMMITTEE (REC)

PO Box 71, Bushenyi, Uganda; Tel: +256 758 096 775

E-mail: kiurec2017@kiu.ac.ug; Web site: www.kiu.ac.ug

INFORMED CONSENT DOCUMENT

Study Title: Factors and common bacterial pathogens associated with post-caesarean wound sepsis at Hoima Regional Referral Hospital

Principal Investigator(s): Dr. Muhumuza Ismael

Qualifications: Bachelor of Medicine and Bachelor of Surgery (KIU-TH).

INTRODUCTION

What you should know about this study:

- You are being asked to join a research study.
- This consent form explains the research study and your part in the study
- Please read it carefully and take as much time as you need
- You are a volunteer. You can choose not to take part and if you join, you may quit at any time. You will not lose any benefits you are entitled to if you do not want to participate or if you decide to withdraw in the middle of the study.

Leave blank (for REC Office only): KIU WC REC Stamp:	For REC Office use only: APPROVAL DATE: APPROVED CONSENT REC VERSION NUMBER: PI's NAME: REC NO:

Brief background to the study

The purpose of this study is to assess common organisms of bacterial origin that are associated with wound infection following caesarean delivery and to identify the associated factors. If you agree to be in this study, I will conduct an interview with you.

The interview will include questions about your socio-demographics, Obstetric and hospital factors. The interview will take about 10 minutes to complete. With your permission, we would also like to a physical examination, take samples from wound if found septic and tape-record the interview and take pictures.

Purpose of the research project:

I do not anticipate any risks to you participating in this study other than those encountered in routine medical examination. There are benefits to you as it helps us manage you better, and also information gathered through your participation may lead to improvement in policy for the promotion of health in this country.

Why you are being asked to participate:

You have been recruited to participate because you fulfill the inclusion criteria in this study. All women that have the criteria for inclusion have been given an equal chance to participate in the study.

Procedures:

Some patients who meet the inclusion criteria will be explained to what the study is about, benefits, confidentiality and autonomy will be allowed. Patient consent will be requested from each participant and if granted, physical examination will be done, if a patient has a discharge on the caesarean wound, will be requested to allow exudate sample from post caesarean wound to be taken for microbiological analysis. Using sterile swab sticks, two samples from each participant will be collected by gently rubbing the sterile swab sticks in the infected site (wound depth) using aseptic technique and immediately replaced inside the swab sticks case. The sterile swab stick will be labeled with each participant's study number and transported to Microbiology laboratory immediately for processing and in case of any

delay, the sample will be stored aerobically in the refrigerator at 4-8°C. In addition, a questionnaire will be administered to each participant to collect information on demographic and known factors that may be related to the development of sepsis in each patient.

Discomforts:

There are minor risks anticipated in this study, the act of taking an exudate swab may cause pain.

Benefits:

The participant shall be educated on the individual associated factors that may result into post-caesarean wound sepsis. This will lead to mass sensitization and guiding policy for management of post-caesarean wound sepsis

Incentives / rewards for participating:

No payment shall be made to you for purposes of participation in this study. Any appreciation given to you in any form should not be considered as part of the research protocol.

Protecting data confidentiality:

You are assured that any information given will not be linked to you directly and your personal details will not be shared with any person. These results shall not be disclosed to anyone without the consent of the research participant.

Protecting subject privacy during data collection:

Data shall be obtained from an enclosed place in the maternity postnatal ward.

Right to refuse / withdraw:

Your participation in the study is purely voluntary, and refusal to participate will involve no loss of benefits that you are entitled.

Leave blank (for REC Office only): KIU WC REC Stamp:	For REC Office use only: APPROVAL DATE: APPROVED CONSENT REC VERSION NUMBER: PI's NAME: REC NO:

What happens if you leave the study?

You are invited to participate in the study. Note that it is your right to accept or not to accept and that your refusal shall not interfere with the services provided to you at Hoima Regional Referral Hospital.

Who do I ask/call if I have questions or a problem?

You may reach the principle investigator through the following contacts:

Principal Investigator Mobile Number Tel: +256-754- 107-353

KIU-TH Research Ethics Committee Tel: +256-758-096-775

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature on this form means

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this study.

Print name of adult Participant	Signature or thumb print of adult participant	Date
Print name of person obtaining Consent (Researcher)	Signature	Date
Full names of witness	Signature or thumb print of witness	Date

Leave blank (for REC Office only): KIU WC REC Stamp:	For REC Office use only: APPROVAL DATE: APPROVED CONSENT REC VERSION NUMBER: PI's NAME: REC NO:

Appendix II: TRANSLATED CONSENT FORM: (RUNYOORO-RUTOORO)

KAMPALA INTERNATIONAL UNIVERSITY (KIU) WESTERN CAMPUS (WC)

RESEARCH ETHICS COMMITTEE (REC)

PO Box 71, Bushenyi, Uganda; Tel: +256 758 096 775

E-mail: kiurec2017@kiu.ac.ug; Web site: www.kiu.ac.ug

KUGARUKAMU KWANGE NKOKUBANSOBOROIRE

Omutwe gwo musomo: Ensonga nobuhuka obuleeta endwara ha mukazi asemezeibwebwamu na zaara omwana omu irwaro lya Hoima Regional Refferal hospital

Omuserulizi mukuru: Dr Muhumuza Ismael

Obukugu: Dokita

OKWANJURA

Ekyosemereire kumanya ha musomo ogu:

- Osabirwe kwegaitaho omumusomo ogwo kuchondoza.
- Enu ebaruha ekusoboora omusomo nekichweka kyawe omumusomo
- Nosabwa okugisoma nobwegendesereza kandi okihe obwiire obukumara
- Olimuyambi. Noyekomeramu butetaba mu musoma gunu kandi nobworayetabamu nosobora kurugamu hokwendera. Tiharabeho buzibu bwoona obworayenda kuleka omusomu ogu.

Leave blank (for REC Office only): KIU WC REC Stamp:	For REC Office use only: APPROVAL DATE: APPROVED CONSENT REC VERSION NUMBER: PI's NAME: REC NO:

Kubaha ebikukwataho omusomo ogu mubigufu

Omugaso gwo'musomo gunu nukwo kumanya oburwaire obuli omubakazi abasemezibwemu abaana no kumanya ebikuleta oburwaire obu. Obworaikiriza okuba omumusomo ogu twija kukukaguza ebikaguzo. Ebikaguzo bijakubamu ebikaguzo ebirukukwataho nkomuntu, ebikaguzo hali iwe omukazi aine enda, nebihabuzo ebirukukwataho irwaro. Ebihabuzo bijakutwara edakika 10 kuhwayo. nitutukusaba kutwikiriza kukukwata eiraka lyawe obworaba nogarukamu ebikaguzo binu tutwale nebisisani byawe

Omugaso gwo kutolereza kunu:

Tinkunihira noija kutunga obuzibu bwoona obwooraba oli mumusomo oihireho ebyo ebya bulikiro . noja kugoboramu habwokuba nikija kutuyamba kunjanjaba kurungi kandi ne bituraba twihire mukuchondoza oku nokwegaitaho kwawe nibiyamba okukurakurana kwengenderwaho eyebyobwomezi.

Habwaki osabirwe kwegaita hamusomo ogu:

Osabirwe okwegaitaho habwokuba oine ebirikwetagisa kuba omumusomo gunu. Abakazi boona abaine ebikwetagisa bahairwe omugisa ogukwingana kwegaita omumusomo gunu.

Nkoku turakora:

Abarwaire abasemerire kutwegaitaho baija kusobororwa omutwe gwo musomo gunu, amagoba, ensita no bugabe bwabo nibwija kwikirizibwa.

Omurwaire twija kumusaba kutwikiriza twihemu amasira omubihoya tugatware nambere bakukebereza tusere obuhuka oburukuleta oburwaire . Nitwija kwongera kukuhabuza ebintu ebirukuretereza ebihoya nobuhuka oburukukwata ebihoya. Omurwaire bwarayanga kumwihaho amasira nitwija kumujanjaba nkabulikiro.

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Ebirukutalibaniza

Nitutebekereza ngu nihaija kubaho obusasi butaito obuturaba nitwihaho amasira

Amagoba:

Abarayetaba mumusomo nitwija kubasomesa ebintu ebirukuletera ebihoya byakwatwa obuhuka. Kinu nikija kuyamba abantu na marwaro okusobora kujanjaba ebihoya ebyabakazi abasemezibwe.

Kusasurwa:

Tiharoho kusasurwa kwona okwija kubaho habwokwegaita omumusomo ogu. Kusima kwona okubarakuha otakutware nka ebi ha bigenderwa byo musomo ogu.

Kwahura Ensita:

Nomanyisibwa ngu ebitukugenda kwiha omumusomo ogu titukwija kubisasanya mu bantu bandi oihireho iwe obworaba oikirize.

Kulinda Ensita obutukuba nitukwihaho ebikutuyamba kutolereza:

Nitwija kukukaguliza mukikaro ekyensita omwirwaro eryabakazi abazaire.

Obugabe okuruga omumusomo gunu rundi okwanga okwetabamu;

Okweigaita mumusomo gunu kwikiriza kwawe kandi nobworayanga kwegaitaho tiwajunanibwe oba tiwatunge kizibu ha magoba agosemerire kutunga.

Ekikubaho obwokuleka omusomo: Oralizibwe okwegaita hamusomo gunu

kandi oli wobugabe kwikiriza rundi kwanga . Kwanga kwawe tikwalemese obujanjabi bwawe aha irwaro lya Hoima Refferal hospital.

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Noha owokwehabuzaho obwokuba oine ebihabuzo orundi ekizibu?

Nosobora kuhika hamurambuzi noraba muri enamba egi; Tel: +256-754-107-353

KIU-TH Research Ethics Committee (Mukulu mukuseruruza) Tel: +256-758-096-775

Kwijuza kwawe habaruha enu nikumanyisa ki?

- Omanyisibwe ha musomo gunu, omugaso gwo musomo, nkokuturakora, amagoba nobuzibu.
- Ohairwe omugisa kukaguza otakaijwize.
- Oikirize kuba omumusomo gunu

....

Ibara lyowayegaisire mumusomo Signature Ekiro

....

Ibara lyomuntu owakusaba kwikirizibwa Signature Ekiro

Leave blank (for REC Office only):	**For REC Office use only:**
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Appendix III: INVESTIGATOR ADMINISTERED QUESTIONAIRRE

Kindly respond to the following questions at will and should you feel any discomfort, you are free to discontinue from the study or leave the question blank.

Individual Factors(health, social and demographic)	
Q1	Age: .....years
Q2	Tribe .....
Q3	Marital status.....
Q4	Address .....
Q5	Weight.....Kg
Q6	Highest attained education level: 0. None [ ] 1. Primary [ ] 2. Secondary [ ] 3. tertiary [ ]
Q7	Height.....in meters
Q8	History of diabetes: 0 No [ ] 1 yes[ ]
Q9	History of hypertension: 0 No [ ] 1 yes[ ]
Q10	History of HIV : 0 No [ ] 1 yes[ ]
Q11	History of TB : 0 No [ ] 1 yes[ ]
Q12	History of cancer : 0 No [ ] 1 yes[ ]
Q13	Family history of cancer : 0 No [ ] 1 yes[ ]
Q14	History of any other chronic illness 0 no [ ] yes [ ] if yes which one: .....
Q15	Employment: a.) farmer b.) Health worker c.) Saloon d.) Teacher e.) Other, specify.....
Q16	Religion 1 catholic [ ] 2. Anglican [ ] 3. Muslim [ ] 4.SDA [ ] 5.Bishaka [ ] 6. Other [ ] If other .....
Q17	How many times do you bathe a day after c/s? .....
Hospital Factors	
Q18	Presence of wound sepsis post- caesarean 0. No [ ] 1. Yes [ ]
Q19	If yes; When was the onset of wound discharge post caesarean.....
Q20	Who performed the surgery? A.) intern b.) Medical officer c.) Senior House Officer d.) Consultant e.) Other specify.....
Q21	Were Antibiotics given before CS? a.)Yes b.) No if yes, mention.....
Q22	Were Antibiotic given after CS? a.)Yes b.)No if yes, list them.....
Q23	Was the patient a referral? a.) yes b.) No if yes, from which health facility?

	.....
Q24	Was antiseptic used? A.) Yes b.) No if yes, list
Obstetric Factors	
Q25	What was the length of Labor? .....in hours.
Q26	Parity.....
Q27	What was the diagnosis/indication of CS? .....
Q28	What type of incision was made on skin? a.) transverse b.) midline
Q29	How many vaginal examinations were made according to partograph? .....
Q30	What type of c/s was performed according to urgency? A.) emergency b.) elective
Q31	What technique of c/s was employed? a.) classical b.) LTCS c.) other
Q32	Were there any pregnancy complication like anemia, PET or APH?
Q33	What is the estimated amount of blood loss during caesarean section?
Q34	How many layers were used to close the uterus? A.) single b.) multilayer
Q35	Premature rupture of membranes 0. No [ ] 1. Yes[ ] If yes; What was the length of time after rupture of membranes before the C/S was performed? .....in hours
Q36	How many prior caesarean sections have you undergone? A.)1 b.) more than 1
	FOR OFFICIAL USE BY THE RESEARCHER
Q37	What is the bacteria isolated from the specimen obtained? .....
Q38	What is the anti-bacterial susceptibility of the bacteria isolated from the specimen obtained.....

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POSTGRADUATE STUDIES & RESEARCH DIRECTORATE (PGSRD)

20th April, 2018

TO

Dr.Muhumuza Ismael
REG. No MMED/3347/153/DU

LETTER OF APPROVAL

This is to certify that the research proposal entitled ***"Factors and Common Bacterial Pathogens Associated with Post-Cesarean at Hoima Regional Referral Hospital"*** was reviewed by the Research Subcommittee of the Board of Postgraduate Studies and Research Directorate of Kampala International University-Western Campus (KIU-WC) in its meeting on 23rd January, 2018 for its Scientific Validity and Ethical appropriateness and was approved subject to minor corrections.

This proposal was finally approved on 20th April, 2018 after the expedited review following the execution of minor corrections. You are required to proceed to KIU Research Ethics Committee for the final approval before data collection.

Wishing you all the best

Signed by:

20 APR 2018

DR.TWINAMATSIKO MEDARD KATONERA
Chairman, Research Sub-Committee **Date/Stamp**

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Website: www.kiu.ac.ug

RESEARCH ETHICS COMMITTEE (REC)

04 JULY 2018

Our ref: **SF201808**

MUHUMUZA ISMAEL
Principal Investigator

APPROVAL OF YOUR PROPOSAL

Submitted Proposal: "FACTORS AND COMMON BACTERIAL PATHOGENS ASSOCIATED WITH POST-CESAREAN WOUND SEPSIS AT HOIMA REGIONAL REFERRAL HOSPITAL UGANDA" Nr UG-REC- 023/201808

Reference is made to the above Protocol, which you submitted to the Research Ethics Committee (REC) for ethical review and approval. It has been noted that all the concerns raised earlier by the Committee, in its meeting of 06th June 2018, have been properly responded to.

This is, therefore, to inform you that your study has been approved; following an Expedited Review. You may now proceed with preparations to implement the research. Please note that this approval is for a period of one year.

As Principal Investigator, you are expected to fulfill the following conditions; which are part of the approval process regarding your study:

1. You are required to register the Protocol with the Uganda National Council for Science and Technology, according to the guidelines of the Council, for final clearance to undertake the research.

2. Any changes/amendments and/or additions to the Protocol, Consent Form and/or Data Collection Tools must be submitted to the REC for review and approval prior to activation of the changes.

3. Reports of unanticipated problems involving risks to participants should be submitted to REC.

4. Only the approved Consent Forms should be used in enrolling participants. For that purpose, therefore, you should retain all signed Consent Forms on file.

KIU RESEARCH ETHICS COMMITTEE(REC)
APPROVED
VALID UNTIL
04 JUL 2019