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POST CAESAREAN SECTION INFECTIONS AT THE
KORLE-BU TEACHING HOSPITAL

BY

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DECLARATION

I hereby declare that, with the exception of the references acknowledged, this thesis is a product of my own investigations and has not been presented in whole or in part to another University for the award of any degree.

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I hereby affirm that the preparation and presentation of this thesis were done in accordance with the guidelines on supervision of thesis laid down by the University of Ghana.

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DEDICATION

I dedicate this thesis to my family.
ABSTRACT

Background: Caesarean section (CS) is one of the most frequent surgical procedures performed on women worldwide. Post CS wound infection is a major cause of prolonged hospital stay, complicating 3% to 15% of CS procedures worldwide. Surgical site infections (SSI) are associated with a maternal mortality rate of up to 3%. With the continuous increase in the rate of CS procedures performed, it is expected that post CS infections will increase in parallel, hence its significance.

Aim: This study aimed at determining the organisms associated with post CS infections and their antimicrobial susceptibility patterns. Risk factors that predispose individuals to infections after CS were also determined.

Methodology: Women who underwent CS procedures at the Korle-Bu Teaching Hospital (KBTH) between April – Dec 2017 were surveyed. Wound swabs were collected from study participants who developed post CS infections within 30 days after surgery for culture and susceptibility tests. Bacterial isolates that were multi drug resistant were further screened for antibiotic resistance markers using PCR methods. The markers assayed included meca and ESBL genes. A structured questionnaire was used to assess risk factors associated with post CS infections.

Results: This study included 907 women who underwent CS, of which 134 developed infections. Post CS infection prevalence was 14.8% (134/907). One hundred and nineteen (119) isolates were cultured from the 134 wound swabs collected. Staphylococcus aureus was the most frequent organism isolated 19.3% (23/119). Coagulase negative Staphylococcus 17.6% (21/119), Escherichia coli 14.3% (17/119) and Pseudomonas aeruginosa 13.4%
were other frequently isolated organisms. Thirteen (13) out of 23 (56.6%) \textit{S. aureus} and 18 out of 21 (85.7%) Coagulase Negative \textit{Staphylococcus} were methicillin resistant. Six (6) out of 17 (35.2%) \textit{Escherichia coli} and 4 out of 12 (33.3%) \textit{Klebsiella species} were ESBLs. CTX-M, TEM and SHV types were the genes encoding ESBL among the Enterobacteriaceae. The most significant risk factors found to increase the risk of developing an infection after CS were labour before CS procedure, length of surgery, use of general anaesthesia and the use of stagnant water as scrub water.

\textbf{Conclusion:} There is a high prevalence of post CS wound infections at the KBTH with a variety of organisms being implicated, predominantly \textit{S. aureus}. It was also found that multi-drug resistant organisms were prevalent. Risk factors associated with post CS infections consisted sociodemographic, obstetric and intuitional factors.
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List of Abbreviations and Acronyms

ANC  Antenatal care

ASEPSIS  Additional treatment (drainage, antibiotics, debridement), Serous discharge, Erythema, Purulent exudate, Separation of deep tissues, Isolation of bacteria, Stay in hospital > 14 days

AST  Antimicrobial susceptibility test

ATCC  American type culture collection

BA  Blood agar

BES  Brazil extended spectrum

bla  Beta-lactamase

CDC  Centre for disease control

CLSI  Clinical laboratory Standards Institute

CoNS  Coagulase negative Staphylococcus

CS  Caesarean section

CTX-M  Cefotaxime hydrolysing capabilities

DNA  Deoxyribonucleic acid

E. coli  Escherichia coli

ESBL  Extended spectrum beta-lactamase

Hep. B  Hepatitis B

HIV  Human immunodeficiency virus
IBC  Integron-borne cephalosporinase
KATH  Konfo Anokye Teaching Hospital
KBTH  Korle-BU teaching Hospital
MA  MacConkey agar
MHA  Muller Hinton agar
MRSA  Methicillin resistant *Staphylococcus aureus*
No.  Number

*P. aeruginosa*  *Pseudomonas aeruginosa*

PER  *Pseudomonas* extended resistance

Prev.  Previous

PROM  Premature rapture of membranes

*S. aureus*  *Staphylococcus aureus*

SDA  Sabouraud agar

SFO  *Seratia fonticola*

SHV  Sulphhydryl variable

SSI  Surgical site infections

STG  Standard treatment Guidelines

SVD  Spontaneous vaginal delivery

TEM  *Temoniera*

TLA  *Tlahuicas Indians*
WHO  World health organisation
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Caesarean section (CS) is an important procedure in elective and emergency obstetric care (Chu et al., 2015). An estimated number of 18.5 million caesarean sections are performed worldwide each year (Gibbons et al., 2010). As facilities expand their surgical capacity, determining the quality of surgical care is essential. However, finding the appropriate indicators are challenging. Caesarean sections like all other surgical procedures are prone to complications including surgical site infections (SSI). Surgical site infections can also be used as an indicator to assess surgical quality (Chu et al., 2011; Petroze et al., 2013; Stewart et al., 2015). Since the 19th century, when the use of antiseptics were introduced into surgical procedures, there has been improvements in the field of microbiology and surgery. Antimicrobials made surgeries safer. Nevertheless, SSIs remained a burden (Gottrup et al., 2005). Surgical site infections are the second most common of type health care associated infections after urinary tract infections (Lewis et al., 2013) However, in sub Saharan Africa, SSIs are the most common healthcare associated infections (HAI) causing 14-30% of all HAI (Nejad et al., 2011; WHO et al., 2018). The emergence of post CS infections depends on the population under study, the infection surveillance criteria being used, and the use of appropriate antibiotic prophylaxis (Gottrup et al., 2005; Jido & Garba, 2012).

Microbes associated with post CS infections can be part of the patients’ normal flora (endogenous sources) or from the environment (exogenous sources); hospital environment, other patients, food, water, health workers, visitors and so on (Schabrun & Chipchase, 2006: Jackie, 2012). Among the many different pathogens that are responsible for these infections, \textit{S. aureus} has been found to be the most common (Mpogoro et al., 2014). With the emergence
of resistant strains like Methicillin Resistant *S. aureus* (MRSA) and Extended Spectrum Beta Lactamases (ESBL), treatment of these wounds can be challenging (Manyahi *et al*., 2014).

Factors, which predispose women to post CS infections like any other SSIs, are multifactorial. These risk factors include prolonged labour, premature rupture of membranes (PROM), multiple vaginal examinations and manual removal of placenta. Co-morbidities such as diabetes mellitus, maternal obesity, and infections like HIV also predispose women to post CS infections. Other variables shown to be associated with SSI rates include hospital environment, age, obesity, steroids use, prolonged hospitalization before surgery and antimicrobial prophylaxis. The peculiar locations of incisions made during CS and their proximity to the perineum, in addition to the immune modulation during pregnancy predispose the surgical site to colonization by infectious microorganisms and coliforms (Killian *et al*., 2001; Olsen *et al*., 2008; Mpogoro *et al*., 2014)

1.2 Problem statement

Surgical Site Infections (SSI) complicate up to 15% of all CS. Not only are these infections associated with significant morbidity but also increase the financial burden of the new mothers, their families and the nation as a whole. This also increases the recovery time after surgery and affects breastfeeding and general mother-baby bonding. Surgical site infections are the most frequent HAI in Sub Saharan Africa (Nejad *et al*., 2011; Mpogoro *et al*., 2014; Chu *et al*., 2015). In spite of the advancement in surgery and infection control measures like improvement of surgical techniques, barrier nursing and antimicrobial prophylaxis, SSI continue to be a major cause of maternal morbidity and prolonged hospital-stay (Savino *et al*., 2005).
The national assessment for emergency obstetric and new-born care conducted by the Ghana Health Service in 2011 stated that caesarean section rate is 7% of live births. At the Korle- Bu Teaching Hospital (KBTH) CS rate is as high as 47% (MOH, 2011; Samba & Mumuni, 2016).

There is scanty information on the prevalence of post CS infections, the microbial profile and the risk factors of these infections. Therefore, there is barely enough evidence to support the choice of antimicrobial prophylaxis and treatments for wound infections following CS.

1.3 Justification

Investigations have been done in other African countries and other parts of the world on post CS infections; its prevalence, microbial profile and risk factors. However, data on this subject is scarce in Ghana. Surgical site infections are a major cause of re-admissions and increase in recovery time after CS at KBTH. It is known that the extended stay of these mothers further complicates the sparse bed space in this hospital but the extent of this problem is not well defined.

This research will bring to light the burden of SSI among women who have undergone CS, the prevalence of post CS infections and the risk factors of these infections at the KBTH. The study also seeks to further investigate the microbial profile, the antimicrobial susceptibility and to genetically identify ESBL and MRSA among the organisms that will be isolated.

It is therefore necessary to do this study to develop an evidence based prevention and treatment protocol for infections arising from CS after surgery. Findings from this work will
not only contribute to existing knowledge but will also inspire further research into this area which shall help to develop a better protocol for other institutions and the entire nation.

1.4 Aim
To investigate surgical wound post CS infections at the KBTH.

1.5 Specific objectives
The specific objectives of this study is to determine the;

- prevalence and risk factors associated with post CS infections.
- aetiological agents and their antimicrobial susceptibility profiles.
- prevalence of methicillin resistant *Staphylococcus* isolates and extended spectrum beta-lactamase producing Enterobacteriaceae associated with post CS infections.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Caesarean Sections

Caesarean section is a surgical procedure, which involves making an incision through the abdomen and uterus of a pregnant woman to deliver her baby. Usually this procedure is performed when delivery through the vagina will put the health of the baby, mother or both baby and mother at risk. Therefore, the World Health Organisation (WHO) recommends that CS should be done only when indicated; that is, if normal delivery will have an adverse effect on mother or baby (Betran et al., 2016; WHO, 2017). Conversely, in recent times, this procedure is performed on request without any medical basis (Stjernholm et al., 2010). The rate of CS has increased considerably over the years (Betrán et al., 2016) and this increase can be attributed to recent development in medicine which makes CS almost risk free (Wiklund et al., 2007). Even though CS is safer due to recent advancements in the field of surgery (Savino et al., 2005), complications like HAI including SSIs are unavoidable.

2.2 Healthcare Associated Infections.

The hospital is a known reservoir of bacterial pathogens because it houses a large number of patients carrying various pathogenic microbes and a large number of susceptible people. As a result, HAI is a significant risk of hospitalisation. It is the most common adverse event, which threatens patients' health all over the world. Healthcare associated infections also known as nosocomial infections or hospital-acquired infections are infections that are contracted by a patient while on admission in a health facility. An infection can only be defined as a HAI if the infection was not present before the patient was admitted and within the incubation period of the infecting pathogen. This term also includes infections acquired by health workers while working and infections which appear within 30 days after discharge of a patient from a health...
Depending on the population under study, the prevalence of HAIs is between 2% and 49% (Flevari et al., 2013; Mbim et al., 2016). Studies have shown that nosocomial infections are a cause of one-third of deaths occurring in hospitals. Most of these deaths are associated with surgical procedures and almost half of the deaths are associated with stay in intensive care units (Gross & Van Antwerpen, 1983).

A survey done by the WHO revealed that only 16% (23/147) of developing countries have a working national surveillance system (Allegranzi et al., 2011). The European Centre for Disease Control and Prevention also reported that, only 26% of the low and middle-income countries in Europe have implemented surveillance systems (ECDC, 2010). This means the real picture of HAI is not well represented globally.

HAI usually affects patients whose immune system is compromised due to age, underlying diseases like Acquired Immune Deficiency Syndrome (AIDS) or medical and surgical treatments. In this modern era, medical and surgical treatments are supported with modern technologies like mechanical ventilatory support, total parental nutrition, intracranial pressure monitoring and innovative surgical techniques (Killan et al., 2001; CDC, 2018). These technological advancements, although useful, have rendered patients more prone to acquiring HAIs. This is so because they employ invasive devices and these devices are generally known to be potential causes of infections. (Maki, 1997; Weinstein & Darouiche, 2001).

Among surgical patients the most common HAI they are likely to encounter are SSIs.
2.3 Surgical Site Infections

Surgical site infections are HAIs occurring at the operated site within 30 days after surgery (Condon et al., 1992). Symptoms considered include pain, erythema, induration, localized tenderness or the presence of a purulent discharge at the surgery site (Wilson et al., 1986). Since 1988 surgical site infections have been classified, as nosocomial infection and are now considered the second most commonly encountered HAI (Garner et al., 1988; Lewis et al., 2013).

The definition of SSIs is not universal and this makes it difficult to compare between hospitals. Some criteria in the United States are subjective; allowing surgeons’ discretion (Gibbons et al., 2010) Nonetheless, in the United Kingdom, only diagnosis of infection after culturing of microorganisms from clinical samples is acceptable (Bowler et al., 2001). Surgical site infections are particularly common following CS and the prevalence ranges between 3 and 15% depending on the study population, the infection surveillance criteria employed and the use of appropriate antibiotic prophylaxis (Chu et al., 2015).

Diagnosis of SSIs are made depending on the presenting clinical signs and symptoms with the help of criteria like the Southampton wound assessment scoring scale, the numerical ASEPSIS criteria and the CDC criteria (Wilson et al., 1986; Garner et al., 1988; Horan et al., 1992;). In a study of SSIs in hospitals in London, it was demonstrated that different methods of diagnosing SSIs gave inconsistent results. It is therefore important, to develop a protocol to make assessment uniform (Wilson et al., 2004).
Forty to fifty percent (40-50%) of SSIs can be prevented by putting systems like appropriate antimicrobial prophylaxis, surveillance and infection control programs in place (Odom-Forren, 2006). A surveillance program conducted in a surgical department in Denmark over a five-year period demonstrated that infection could be markedly reduced when infection control measures are taken seriously (Schneeberger et al., 2002). Since 2004, all the departments of health in the United Kingdom made it a requirement for all National Health Service trusts with orthopaedic surgery departments to undertake surveillance for at least three months every year. This has reduced the rate of SSIs since 2004. It can be inferred that surveillance is a vital instrument for preventing the surge of SSIs. Surveillance and infection control does not only reduce the number of infections but also makes prescribers aware of the trend of organisms causing these infections. Knowledge of common aetiological agents and their antimicrobial susceptibility patterns helps them make informed choices during wound dressing, administration of antibiotic prophylaxis and treatment (HPA, 2007).

2.3.1 Classification of Surgical Wounds

Prophylaxis and the extent of wound care targeted at preventing SSIs would depend on the type of surgical wound (Gillespie et al., 2015). Surgical wounds are classified into clean, clean/contaminated, contaminated and dirty. This classification has been the basis for infectious risk assessment, perioperative protocol development and surgical decision-making.

Clean wounds are surgical wounds, which are not infected or contaminated. Surgeries involving these wounds do not enter the respiratory, genital, alimentary or urinary tracts.

Clean-contaminated wounds are operative wounds that involve the respiratory, genital, alimentary or urinary tracts and are done under controlled conditions without contamination.
Though these wounds may not show any inflammation or infections, the risk of developing an infection is high.

A surgical wound is considered as a contaminated wound when a surgical procedure, which should be clean, is compromised due to a break in the sterile surgical procedure. In the case of CS, procedures can become contaminated when there is a spillage from the gastrointestinal tract.

Dirty wounds however are associated with old traumatic wounds with retained devitalized tissue and wounds involving existing clinical infection or perforated viscera.

Elective CS wounds are considered clean wounds. Emergency CS wounds on the other hand are sometimes clean-contaminated wounds because some of these surgeries are performed on patients who are in labour and have had vaginal examination. In these instances, the amniotic membrane is absent creating a communication between the genital tract and the uterus. In cases where there is obstructed labour and developed frank chorioamnionitis, the wound is classified as a contaminated wound. This is because these surgeries involve infected amniotic fluid (Condon et al., 1992).

The risk of SSI could be dependent on whether the surgery is clean, clean-contaminated or dirty-contaminated. Caesarean section procedures are clean. Therefore, posing the least risk of post-surgery infection on this note. It is however possible to have CS procedures being clean-contaminated (Mangram et al., 1999).
2.4 Risk factors for Post Caesarean Section Infections

Women who are pregnant or recently been through pregnancy are generally at a risk of developing infections because pregnancy compromises immune response to infections (Kourtis et al., 2014). This means that women who have undergone CS already have an intrinsic risk of contracting SSI. Risk factors for SSI can be classified into patient (maternal)-associated risk factors and Institutional risk factors. The rates of post CS infections differs from population to population. These differences are usually due to the inherent differences between the individual members of the population, the methods used to monitor and identify cases and the presence or absence of the appropriate antibiotic prophylaxis (Mitt et al., 2005; Schneid-Kofman et al., 2005).

Some risk factors known to predispose women who have undergone CS include manual extraction of placenta, premature birth, excess vaginal examinations, premature rapture of membranes, prolonged labour co morbidities like gestational diabetes, HIV, severe anaemia among others (Björklund et al., 2005; Farret et al., 2015). It has also been established that the use of appropriate antibiotic prophylaxis is very useful in the prevention of the post CS infections. In some health facilities, antibiotics are administered only after the umbilical cord is clamped because of the impact of antibiotic use among neonates while other institutions administer these antibiotics during the surgical procedure (Killian et al., 2001; Baaqeele & Baaqeele, 2013).

Apart from individual host factors mentioned above, other factors for post CS infections, include length of operation and the extent of microbial contamination of the surgical wound, the possibility of ineffective sterilization of surgical instruments, unavailability of improved
operation theatre ventilation systems and length of preoperative procedures (shaving and antisepsis of the operation area). (Spagnolo et al., 2013).

2.5 Aetiology of Post Caesarean Section Infections

In the late 1940s and 1950s major pandemics caused by *S. aureus* phage type 94/96 occurred in the United States (APMIS, 1996). Before this organism, the major cause of nosocomial infections was *Streptococci* species. Then in the 1970s, there was an emergence of *Pseudomonas* species and *Enterobacteriaceae*. Gram-negative bacilli continued to be prevalent until the late 1980s when the Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* began to surface. By 1996, the list of organisms causing HAIs increased. The list included three species: gram-positive organisms (*S. aureus*, Coagulase-negative *Staphylococci* and *Enterococci*) and four gram-negative species *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These organisms accounted for over 60% of all nosocomial infections during that time (Weinstein, 1998; Pearson, 2009).

The trend has not changed much over the years. *S. aureus* is still reported as the most frequently isolated pathogen causing HAIs. The other frequently isolated organisms are the coagulase-negative *Staphylococcus*, *Enterococcus* species, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Streptococcus* species, *Proteus mirabilis*, and *Candida albicans* ( Richards et al., 2000; Jido & Garba, 2012; Khan et al., 2017)

The microbiological profile of post CS infections at the KBTH is not well established. However, a study conducted by Egyir et al. (2013) at the Child Health and surgical
departments of the KBTH, reported the carriage prevalence of *S. aureus* among inpatients and hospital staff to be 13.9% and 23.3% respectively. Methicillin resistant *S. aureus* was detected in 1.3% of inpatients and none among hospital staff (Egyir *et al.*, 2013). This study therefore suggests that *S. aureus*, which is the most common pathogen isolated from inpatients and staff of the KBTH is likely to be the most common pathogen causing SSI at the KBTH.

### 2.6 Antimicrobial Agents Against Post Caesarean Section Infections

Antibiotic prophylaxis remains a major tool for prevention of SSI worldwide reducing the risk by up to 40%. This is best achieved when the antibiotic is given at induction of anaesthesia to ensure tissue saturation before the incision is done (Pearson, 2009). Prophylaxis for CS in many settings is limited to emergency CS and is limited to the single dose at induction of anaesthesia. Second generation cephalosporin is usually the first line antibiotic for surgical prophylaxis in most of the studies reviewed (Geroulanos *et al.*, 2001; Owens *et al.*, 2009).

At the KBTH, antibiotics are administered routinely to all patients undergoing CS and the first dose is given at induction of anaesthesia. The first-line antibiotics of choice are the amoxicillin-clavulanic acid combination (co-amoksiclav) and metronidazole, and for patients with established chorioamnionitis, hypersensitivity to the penicillin, renal impairment and other contraindications to the penicillin, second and third generation cephalosporin (cefuroxime or ceftriaxone) combined with metronidazole are the antibiotics of choice.

Penicillins (specifically the beta-lactam sub-class) and lincosamides (Clindamycin) remain the first line antibiotics of choice for infected surgical wounds while culture results are
awaited. One of the objectives of this study therefore is to determine the microbial profile of organisms isolated from post CS at the KBTH and their antimicrobial susceptibilities, which will guide clinicians in the administration of first-line broad-spectrum antibiotics to patients who develop SSI while awaiting wound swab culture results.

2.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility Testing (AST) is an important test in the microbiology laboratory which informs the susceptibility of microorganisms to specific antibiotics. Isolation and identification of causative organisms of an infection such as SSI is not enough for a clinician to prescribe antibiotics. AST is therefore, a relevant test, which should ideally precede the administration of antibiotics. In this way, antibiotics will be given rationally and appropriately to patients. However, these tests usually take some days and it will not be realistic to wait for these results in all cases before beginning antibiotic therapy. In addition to confirming empirical antibiotic regimen and determining the most appropriate antibiotic to prescribe for each patient with an infection, it is a tool to detect possible resistance of common microbes to specific antibiotics (Jorgensen et al., 2009).

The most widely used AST methods are broth micro dilution or rapid automated instruments and devices. There are manual methods that are relatively cheaper and flexible to use. These are disk diffusion tests and gradient diffusion tests. Some of these tests are qualitative; detecting only whether isolates are susceptible, intermediate resistant or completely resistant. Others are quantitative; determining the minimum inhibitory concentration of an antimicrobial agent with respect to standard isolates (Jorgensen et al., 2009; Leekha et al., 2011; Syal et al., 2017).
Antimicrobial resistance is the ability of a microbe to survive and multiply in the presence of an antimicrobial agent that would normally inhibit or have a cidal activity against the microbe. Resistance to antimicrobial agents is not a new phenomenon but in recent years, the increase in antibiotic use has increased of antimicrobial resistance. Over the past century many pathogens have developed multiple drug resistance (Alekshun & Levy, 2007). Microbes demonstrate two main types of resistance. These are intrinsic resistance and acquired resistance. When an organism is resistant to an antimicrobial agent before its first introduction to the agent, that organism is said to be intrinsically resistant. Acquired resistance on the other hand is refers to an organism that was originally but susceptible on subsequent introduction to the antimicrobial agent becomes resistant. Microbes can resist the antimicrobials through preventing intracellular access, increase removal of the antimicrobial agent through efflux pumps, modifying the antimicrobial agent through enzymatic breakdown or modifying the target site of the agent in the bacterial cell making the antimicrobial agent ineffective. The emergence of resistance makes treatment of infections difficult. This can pose serious public health threats because it severely limits the therapeutic options for the treatment of infections (Munita & Arias, 2016).

2.7.1 Disk Diffusion Method of Antimicrobial Susceptibility Testing

Disk diffusion method is the qualitative test used to determine the susceptibility of microbes to antimicrobial agents. It is a simple and well-standardized test where a plate of Muller Hinton agar is streaked with a control strain and a test strain at three different angles to give a semi confluent growth. Up to 12 already commercially prepared, fixed concentration paper antibiotic disks are placed on the inoculated agar surface and incubated for 18-24 hours at 37°C before results are taken. Depending on the size of the agar plate, the number of antibiotic disks are different (150 mm diameter: ≤12 disks). The diameter of zones of
inhibition of growth around each of the antibiotic disks are measured to the nearest millimeter. The diameter is related to the susceptibility of the isolate and the rate of diffusion through the agar medium. The diameters of each zone are interpreted using the criteria published by CLSI. Since disk diffusion method is quantitative, the results obtained are recorded as susceptible, intermediate or resistant (Nijs et al., 2003).

The advantages of disk diffusion method are that it is simple to do and does not require any special equipment therefore inexpensive. The results provided are categorical and easy to interpret and selecting disks for testing is flexible. On the other hand, the disadvantage of this method are the lack of mechanization and automation, which makes it is easy to make errors that will alter the results (Coorevits et al., 2015). Not all fastidious bacteria can be accurately tested by this method but the disk diffusion test has been standardized for testing streptococci, *Haemophilus influenzae* and *Neisseria meningitidis* using specialized media, incubation conditions and specific zone sizes (CLSI, 2016).

### 2.8 Molecular Mechanisms of Drug Resistance

The mechanisms microbes use to outsmart antimicrobial agent outdate and outnumber the antibiotics available. Antibiotic resistance is usually attributed to chromosomal mutations but most of the resistance mechanisms are from extra chromosomal elements like plasmid, transposons and integrons found in the environment of microbes. There are intrinsic mechanisms of resistance such as efflux pumps that are not associated to these extra chromosomal elements (Bennett, 2008).
The production of penicillinase enzyme by *Staphylococcus* was the first notable resistance mechanism against antibiotics. Afterwards, many other mechanisms have led to the emergence of multidrug resistance (Barber, 1947). These include production of penicillin-binding proteins (PBPs), mutated drug target sites, enzymatic modification of drugs, altered permeability of bacterial membrane and boosted expression of efflux pumps (Heesemann, 1993). The most troublesome multidrug resistant organisms of recent times include; *Actinobacter baumaii*, *P. aeruginosa*, *E. coli* and *K. pneumonia* producing extended-spectrum beta-lactamases (ESBL), Vancomycin resistant enterococci (VRE), methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus*, extensive drug-resistance (XDR) *Mycobacterium tuberculosis* and so on (Todar, 2012).

### 2.8.1 Extended spectrum beta-lactamase Enterobacteriaceae

Extended spectrum beta-lactamases (ESBLs) are hydraulic enzymes, which are produced by some drug resistant Enterobacteriaceae. Infections caused by ESBLs are more difficult to treat because they tend to be resistant to several antibiotics. Most common ESBL producing organisms are *E. coli* and *K. pneumoniae* (Paterson & Bonomo, 2005). They are also produced by *Actinobacter baumannii* and *P. aeruginosa* (Jacoby & Munoz-Price, 2005). Extended spectrum beta-lactamases are resistant to most beta-lactam antibiotics like penicillins, cephalosporins and monobactam aztreonam. These enzymes act by cleaving the beta-lactam ring and inactivating them. Genes for ESBL production can be carried either by chromosomes or by plasmids, which are able to move between bacterial populations.

The first plasmid-mediated ESBL organism was discovered in the 1960s in Greece. It was called ‘Temoniera’ (TEM), the patient from whom it was isolated (Bradford, 2001).
Afterwards another closely related enzyme was found and named TEM-2. The later and the former only differed by a single amino acid with a resulting change in the isoelectric point of the enzyme. Since then other ESBL gene variants have been discovered with the most common variants being the SHV, TEM, and CTX-M genotypes (Rupp and Fey, 2003). Other clinically significant types are BES-1, IBC, PER, SFO, TLA, VEB (Jacoby & Munoz-Price, 2005).

The prevalence of ESBLS are underestimated because it is often challenging to detect ESBLs in clinical laboratories (Bradford, 2001; Ben-Ami et al., 2009) and the prevalence of ESBLs varies across the world. The prevalence of ESBLs in Europe and America have been reported to be less than 20% (Cantón et al., 2008; Hoffman-Roberts et al., 2016) while in Asia and Africa it has been reported to be as high as 60% (Storberg, 2014; Quan et al., 2017). In Ghana, the prevalence of ESBL is almost 50% and the common gene variants encoding ESBL production are SHV, TEM, and CTX-M genotypes (Feglo et al., 2013; Obeng-Nkrumah et al., 2013). Extended spectrum beta-lactamase producing organisms have been reported to be associated with post CS infection and other SSI (Dhar et al., 2014; Jolivet et al., 2018).

2.8.2 Methicillin resistant Staphylococcus aureus

Methicillin resistant S. aureus is a genetically distinct strain of S. aureus. This strain is not only resistant to methicillin but also resistant to beta-lactam antibiotics like cephalosporins and carbapenems due to the acquisition of a low affinity penicillin binding protein (PBP2a/PBP2) encoded by mecA gene (Lowy, 2003). Strains of MRSA were identified not long after methicillin was introduced into clinical practice. The first outbreak of MRSA happened in
Europe in the early 1960s (Jevons, 1961). Since then, strains of MRSA and methicillin resistant CoNS have spread worldwide. Methicillin resistant *S. aureus* is a common aetiological agent responsible for HAI with 25% of *Staphylococcus* strains in the USA being methicillin resistant (David & Daum, 2010). There is limited records on MRSA in Africa. In a multicentre study involving five major African towns, a prevalence of 15% was reported (Breurec *et al.*, 2011). In Ghana, the prevalence of MRSA is 3% (Egyir *et al.*, 2014). Methicillin resistant *S. aureus* known to cause HAI are also resistant to erythromycin, clindamycin, fluoroquinolones and tetracycline. Several studies have reported high (20-100%) prevalence of MRSA from SSI including post CS infections (Metri *et al.*, 2011; Saleem *et al.*, 2015). Isolation of MRSA from surgical sites has been associated with delayed wound healing (Grimble *et al.*, 2001).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

Samples were collected from the maternity wards of the Korle-Bu Teaching Hospital (KBTH). Korle-Bu Teaching Hospital is a 2000-bed tertiary hospital which serves both adult and paediatric populations of over 3 million in the Greater Accra Region. The Maternity unit is under the Department of Obstetrics and Gynaecology. This department provides 240 and 114 beds for Obstetrics and gynaecology, respectively. The Obstetrics and Gynaecology Department takes care of women from antenatal, delivery to post-natal care.

In addition to dealing with cases of normal delivery, the Obstetrics and Gynaecology Department have two theatres in which both emergency and elective obstetrics and gynaecology surgeries including CS are conducted.

Wound swabs collected from the Obstetrics and Gynaecology Department were processed at the Medical Microbiology Department of the School of Biomedical and Allied Health Sciences, Korle-Bu.

3.2 Ethical Consideration

Ethical approval was obtained from the Ethics and Protocol Review Committee of the College of Health Sciences of the University of Ghana before the study was carried out (Protocol identification number: CHS-Et/M.10-P2.6/2017-2018). Written informed consent was obtained before study patients were enrolled. All study details were explained to participants before requesting for their consent and subsequent enrolment into the study.
Information provided to participants included the risk, benefits and the right to refuse or withdraw participation from the study at any time. Study participants were assured that enrolment into the study is voluntary and that refusing to participate in the study would not jeopardize their clinical care and management. Participants were informed that there would be no financial incentives for participating in this study.

3.3 Study Design and Study Population

This research was across sectional study, which included women attending the Maternity Unit, who have undergone CS, and have consented to participate in the study. Out of these women those who had infections at the surgery site were recruited for microbiological studies. The diagnosis of infection was based on the criteria stated by the Centre for Disease Control and Prevention (CDC, 2015).

Women who come to the Maternity Unit for emergency situations and those who are unconscious were excluded. Those who were already being treated with antibiotics for diagnosed post CS infections but not the routine prophylaxis were excluded from the antimicrobial analysis.

3.4 Sample Size Determination

The minimum sample size required for this study was 383 using the Cochrane formula for sample size calculation in a cross-sectional study formula;

$$n = \frac{z^2 \times p \times q}{e^2},$$

where, $$q = (1-p)$$

$$z =$$ standard normal variance =1.96 at 95% confidence interval
p= prevalence from literature (47%) (Samba and Mumuni, 2016).

e= absolute standard error= 0.05

3.5 Sample Collection and Processing

Recruitment of participants and sampling was done between April and December 2017 at the maternity ward of the KBTH. Demographic and clinical data were extracted from the patients’ folders using a structured questionnaire. Wound swabs from the infected site of the mothers were collected using a sterile cotton swab and transported on ice to the Medical Microbiology Department, School of Biomedical and Allied Health Sciences, and analysed within 24 hours to maximize the chance of isolating the causative organism.

Swabs were inoculated onto blood agar (BA), chocolate agar (CA) and MacConkey agar (MA) plates. The BA and MA plates were incubated at 37°C for 24 hours in an aerobic condition. The CA plates were incubated at 37°C for 24 hours in a microaerophilic condition. Swabs were additionally inoculated onto sabouraud dextrose agar (SDA) and incubated at 25°C for 3days.

Identification of the isolates were done by gram staining and by the determination of colonial morphology on the plates. Standard biochemical tests were performed as described by Steinbach (2001) to identify the species involved.
3.6 Antimicrobial Susceptibility Testing

Antibiotic susceptibility tests were done using Kirby Bauer’s disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2017. A 0.5 McFarland equivalent suspension of organisms was inoculated on Muller-Hinton agar (MHA) (Oxoid, Hampshire, England) plate as described in the CLSI recommendations (Patel, 2017) using a sterile cotton swab. The antibiotic discs were paced on the agar, left for 15 minutes to set and incubated aerobically for 20-24 hours at 37°C. The antibiotic discs used were Penicillin (10 μg), Tetracycline (30 μg), Cefoxitin (30 μg), Cotrimoxazole (1.25/23.75 μg), Erythromycin (15 μg), Amoxiclav (20/10 μg), and Gentamicin (10 μg) for gram positive organisms and Piperacillin Tazobactam (100/10 μg), Amikacin, Ceftriaxone (30 μg), Ceftazidime (30 μg), Ciprofloxacin (5 μg), Norfloxacin (10 μg) and Gentamycin (10 μg) for gram-negative organisms (BD BBL™ Sensi-Disc Antimicrobial Susceptibility Test Disc).

Cefoxitin (30 μg) was used to screen phenotypically for Methicillin resistance among *Staphylococcus* species. Cefotaxime (30 μg) and Cefotaxime with Clavulanic acid (30 μg/10 μg) were used to screen phenotypically for Extended Beta-Lactamase production among Enterobacteriaceae. All antimicrobial susceptibility results were interpreted according to CLSI guidelines (2017). Control strains used were *S. aureus* ATCC 25923 (American Type Culture Collection) for gram-positive organism and *E. coli* ATCC 25922 *P. aeruginosa* ATCC 27853 (for carbapenems) for gram-negative organisms.

Phenotypically positive Methicillin Resistant *Staphylococcus* isolates and Extended Beta-Lactamase producing Enterobacteriaceae were further screened by molecular methods.
3.7 Bacterial DNA Extraction.

Multidrug resistant isolates, which were phenotypically positive for the Cefoxitin test (S. aureus and coagulase negative Staphylococcus) and double-disc diffusion test, were used for the molecular analysis. Bacterial DNA extraction was done using the Quick-DNA™ Fungal/Bacterial Miniprep Kit. Beta-mercaptoethanol was added to a genomic lysis buffer to optimise performance. Overnight culture of bacterial cells were uniformly suspended in isotonic buffer (Phosphate Buffer Saline) and transferred into ZR BashingBead™ Lysis Tubes. Afterwards Bashing Bead™ Buffer was added to the tube and the ZR BashingBead™ Lysis Tube were centrifuged at 10000× g for one minute.

The supernatant was transferred to Zymo-Spin™ III-F in a collection tube and centrifuged for one (1) minute. Genomic Lysis buffer was added to the filtrate in the collection tubes. The genomic buffer-filtrate suspension was transferred into Zymo- Spin TM IIIC Column in a collection tubes and centrifuged at 10000× g for one (1) minute. The flow was discarded through the collection tubes and the previous step repeated. DNA Pre-Wash Buffer was added to the Zymo- Spin TM IIIC Columns and centrifuged at 10000× g for one (1) minute. A washing buffer (g-DNA Wash Buffer) was added to the Zymo- spin TM IIIC Column and centrifuged at 10000g× g for one minute. The Zymo-spin TM IIC Columns were transferred to a clean 1.5 ml micro centrifuge tube. DNA Elution Buffer was directly added to the column matrix and centrifuged 10000× g for 30 seconds to elute the DNA.

3.7.1 PCR confirmation of mecA gene among Staphylococcus isolates.

PCR was performed to detect mecA genes which is a confirmation of mecA mediated resistance to methicillin among Staphylococcus isolates. The PCR was done using the method described by Pournajaf et al. (2014). The positive and negative controls used were
methicillin-resistant *S. aureus* ATCC 33591 and methicillin-susceptible *S. aureus* ATCC 25923

Table 3.1 *mecA* mediated methicillin resistance PCR Primers and cycling conditions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5’-3’)</th>
<th>T°C*</th>
<th>PCR cycles</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mecA</em></td>
<td>FP: TCCAGATTACAACCTCACCGGG</td>
<td>68°C for 30 seconds</td>
<td>30</td>
<td>(Salaam-dreyer, 2010)</td>
</tr>
<tr>
<td></td>
<td>RP: CCACCTCTATCTTTGTAACG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T°C*, activation temperature; polymerase chain reaction

### 3.7.2 PCR confirmation of extended spectrum beta-lactamase encoding genes.

Extended Spectrum Beta-lactamase encoding genes, *bla*<sub>SHV</sub>, *bla*<sub>OXA-2</sub>, *bla*<sub>OXA-10</sub> and *bla*<sub>CTX-M</sub> groups 1, 2 and 9 were PCR screened in *E. coli* and *K. pneumoniae* isolates that were phenotypically positive. Table 3.2 shows the primer sequences and reaction conditions used. The reaction mix contained 2μL of template DNA, 12.5μL 2× Multiplex mastermix (Inqaba, South Africa), 2.5μL of 10× reverse and forward primer, and 7.5μL of DNAse /RNAse free water (Inqaba, south Africa). Previously characterised strains of specific ESBL genes were used as positive controls. *E. coli* ATCC 25922 was used as a negative control. All PCR protocols included an initial denaturation at 94°C for 15 minutes, 94°C for 30 seconds for 30 cycles and 63°C for 90 seconds. 72°C for 60 seconds and a final extension at 72°C for 10 minutes, with a final hold at 6°C. Multiplex PCR was performed for *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-2</sub> genes at 94°C for 15 minutes and then for 27 cycles for 94°C for 30 seconds, 50°C for 90 seconds, 72°C for 60 seconds and the final extension at 70°C for 10 minutes, with the final hold at 6°C in a thermal cycler. Isolates negative for *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-2</sub> genes were examined for *bla*<sub>CTX-M-9</sub> genes. For *bla*<sub>CTX-M-9</sub>, PCR was done at 94°C for 15 minutes and then for 27 cycles of 94°C for 30 seconds, 50°C for 90 seconds, 72°C for 60 seconds and the final
extension at 72°C for 10 minutes, with a final hold of 6°C. Another Multiplex PCR was performed for \textit{bla}_{SHV}, \textit{bla}_{OXA-2} and \textit{bla}_{OXA-10} genes at 94°C for 15 minutes and then for 30 cycles for 30 seconds, 63°C for 90 seconds, 72°C for 60 seconds, and final extension at 72°C for 10 minutes, with a final hold at 6°C.
Table 3.2 Primers and cycling conditions for ESBL confirmation by PCR

<table>
<thead>
<tr>
<th>Primer (Gene Variant)</th>
<th>Primer sequence (5’-3’)</th>
<th>T°Ca</th>
<th>PCR cycles</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla</em>TEM (918bp)</td>
<td>FP: GTATCCGCTCATGAGACAATAAACCCTG&lt;br&gt;RP: CCAATGCTTAATCAGTGAGGCAACC</td>
<td>63°C for 90 seconds</td>
<td>30</td>
<td>(Oduro-Mensah et al., 2016)</td>
</tr>
<tr>
<td><em>bla</em>SHV (842bp)</td>
<td>FP: CGCCTGTGTATATCTCCCTGTAGCC&lt;br&gt;RP: TTGCCAGTGCTCAGCG</td>
<td>63°C for 90 seconds</td>
<td>30</td>
<td>(Bhattacharjee et al., 2007)</td>
</tr>
<tr>
<td><em>bla</em>OXA-2 (330bp)</td>
<td>FP: GTTAACAGGGGCTTTGCAGG&lt;br&gt;RP: TGCACGAGTATCCAGGTTCG</td>
<td>63°C for 90 seconds</td>
<td>30</td>
<td>(Bhattacharjee et al., 2007)</td>
</tr>
<tr>
<td><em>bla</em>OXA-10 (655bp)</td>
<td>FP: ATGAAACATTTGCGCATTAGTA&lt;br&gt;RP: ACACCAGGATTTGACTCAGTTCC</td>
<td>63°C for 90 seconds</td>
<td>30</td>
<td>(Zafer et al., 2014)</td>
</tr>
<tr>
<td><em>bla</em>CTX-M-1 (940bp)</td>
<td>FP: GACAGACTATTTGTTGTAGGTTACCG&lt;br&gt;RP: CCGTTTCCSCTATTACAAA</td>
<td>50°C for 90 seconds</td>
<td>30</td>
<td>(Shibata et al., 2006)</td>
</tr>
<tr>
<td><em>bla</em>CTX-M-2 (253bp)</td>
<td>FP: ACAGTTGACGCTGCTTGAAG&lt;br&gt;RP: TCAGAAACCGTGGATTACGA</td>
<td>50°C for 90 seconds</td>
<td>30</td>
<td>(Shibata et al., 2006)</td>
</tr>
<tr>
<td><em>bla</em>CTX-M-9 (860bp)</td>
<td>FP: ATGATTGACAAAGAGAGAGAC&lt;br&gt;RP: ATGATTACTCGCGCTGAACG</td>
<td>50°C for 90 seconds</td>
<td>30</td>
<td>(Shibata et al., 2006)</td>
</tr>
</tbody>
</table>

* T°Ca, activation temperature; polymerase chain reaction
3.7.3 Gel electrophoresis.

All PCR products were analysed by horizontal gel-electrophoresis in a 2% (weight/volume) self-made agarose gel (SeaKem® GTG® Agarose, Lonza). Gene Ruler 100bp DNA Ladder Plus (Fermentas, Germany) was diluted 1:10 with Mili-Q® water as size marker. Amplification products (5μl) were diluted 1:4 with water and 0.2% loading dye. Gels were run at 50 Volts for 1 hour plus 15 minutes and stained with Gel Red (Bio-Rad) for 30 minutes. The gels were photographed by use of Ultra violet trans-illuminator and digital capture system.

3.8 Data Analysis

Study data (questionnaire and antimicrobial susceptibility results (AST)) was entered into a Microsoft Excel sheet and exported to Package for Social Sciences (SPSS) V23. Sociodemographic variables, obstetric variables and AST results were described using frequencies and percentages. Furthermore, chi square test (contingency tables) was used to determine the association of various variables to post CS infection. Multiple logistic regression was then used to determine the significant predictors of post CS infections.

A confidence interval used for the analysis was 95% and P value below 0.05 was considered (P<.0.5) significant.
CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence and Sociodemographic Data of Post CS Infections at the KBTH

During the eight-month survey at the KBTH, nine hundred and seven (907) women were surveyed. Out of them, 134 representing 14.8% developed post CS infection within 30 days after surgery.

Table 4.1 shows the Sociodemographic information of the women who participated in the survey. The average age of the study participants was 27 years and majority of the women were between the ages of 25 and 34. Most of the women who had post CS infections were either overweight (42.5%) or obese (35.8%). Over 60% of the women who were infected were married. Only a few of the study participants (7.5%) had no formal educational background and almost 45% of these women had at least a senior high school education. More than 30% of them had up to Junior high school education and of all the participants in this study, less than 2% were housewives or unemployed. On the other hand, most of the women (48%) were traders or farmers. The women who had post CS infection with a history of alcohol consumption were 35 (26.2%). Also only 89 of the women who took part in the survey had a history of smoking, and none of these women developed post CS infection during the survey.

Age, BMI, marital status, occupation and alcohol consumption had no association with the development of post CS infection. However, level of formal education and smoking showed no significant association with the development of post CS infection.
Table 4.1 Sociodemographic data of participants who had CS at the KBTH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infected N=134 (%)</th>
<th>Not infected N=773 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>11 (8.2)</td>
<td>20 (2.6)</td>
<td>0.041*</td>
</tr>
<tr>
<td>20-24</td>
<td>22 (16.4)</td>
<td>75 (9.7)</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>40 (29.9)</td>
<td>241 (31.2)</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>41 (30.6)</td>
<td>253 (32.7)</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>20 (14.9)</td>
<td>184 (23.8)</td>
<td></td>
</tr>
<tr>
<td>BMI group</td>
<td></td>
<td></td>
<td>0.027*</td>
</tr>
<tr>
<td>Underweight</td>
<td>4 (3.7)</td>
<td>17 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>24 (17.9)</td>
<td>240 (31.0)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>57 (42.5)</td>
<td>198 (25.6)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>48 (35.8)</td>
<td>318 (41.1)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td>0.005*</td>
</tr>
<tr>
<td>Married</td>
<td>90 (67.2)</td>
<td>632 (81.8)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>33 (24.6)</td>
<td>115 (14.8)</td>
<td></td>
</tr>
<tr>
<td>Co-habiting</td>
<td>9 (6.6)</td>
<td>26 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Divorced/Separated</td>
<td>2 (1.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Highest level of formal education</td>
<td></td>
<td></td>
<td>0.421</td>
</tr>
<tr>
<td>No education</td>
<td>4 (3.3)</td>
<td>64 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>13 (9.8)</td>
<td>80 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Junior High School</td>
<td>46 (34.1)</td>
<td>308 (39.8)</td>
<td></td>
</tr>
<tr>
<td>Senior High School</td>
<td>31 (23.1)</td>
<td>154 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Tertiary and above</td>
<td>40 (29.5)</td>
<td>167 (21.6)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Artisan</td>
<td>13 (9.8)</td>
<td>189 (24.5)</td>
<td></td>
</tr>
<tr>
<td>Trader/farmer</td>
<td>48 (36.1)</td>
<td>388 (50.2)</td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>42 (31.1)</td>
<td>127 (16.4)</td>
<td></td>
</tr>
<tr>
<td>Skilled worker</td>
<td>18 (13.1)</td>
<td>41 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>4 (3.3)</td>
<td>20 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Housewife/unemployed</td>
<td>9 (6.6)</td>
<td>8 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Yes</td>
<td>35 (26.2)</td>
<td>36 (4.6)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>99 (73.7)</td>
<td>737 (95.4)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0.0)</td>
<td>89 (0.5)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>134 (100.0)</td>
<td>684 (99.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant; BMI- Body mass index
4.2 Obstetric History of Women who had Caesarean Section at the KBTH

Table 4.2 shows the obstetric history of the participants surveyed. The average gestational age was 37 weeks. Majority of the cases were emergency CS and in labour. About 83% of the infected women had emergency CS. Out of the women who participated in the study, 70% (542) had previously delivered through CS. More than 50% of the women who were infected had never had CS. Over 98% of all the participants of the study attended ANC and more than 50% of them attended ANC at a clinic elsewhere.

Less than 4% of all the women surveyed were diabetic and close to 7% of those who were infected were diabetic. None of the infected women had syphilis but approximately 5% of them were HIV and Hep B positive. Among the group of women who developed post CS infection more than 30% had PROM and 5% had chorioamnionitis.

Table 4.2 also shows that previous mode of delivery, number of ANC visits, urgency of the procedure (elective or emergency CS), augmentation of labour, type of anaesthesia, number of days on admission before surgery, length of surgery and number of hours before surgery had an association with the development of post CS infection.
Table 2.2 Obstetric history of participants who had CS at the KBTH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infected N=134 (%)</th>
<th>Not infected N=773 (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prev. mode of delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean</td>
<td>57 (42.5)</td>
<td>485 (62.8)</td>
<td>0.028*</td>
</tr>
<tr>
<td>SVD/First delivery</td>
<td>77 (57.5)</td>
<td>288 (37.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Antenatal attendance</strong></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>131 (97.8)</td>
<td>761 (98.4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3 (2.2)</td>
<td>12 (1.6)</td>
<td></td>
</tr>
<tr>
<td><strong>No. of ANC visits</strong></td>
<td></td>
<td></td>
<td>0.003*</td>
</tr>
<tr>
<td>&lt;4</td>
<td>31 (23.1)</td>
<td>67 (8.8)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>103 (76.9)</td>
<td>694 (91.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Level of ANC attendance</strong></td>
<td></td>
<td></td>
<td>0.986</td>
</tr>
<tr>
<td>Maternity home</td>
<td>9 (6.7)</td>
<td>53 (6.9)</td>
<td></td>
</tr>
<tr>
<td>CHPS compound</td>
<td>2 (1.5)</td>
<td>19 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Clinic</td>
<td>72 (53.7)</td>
<td>408 (52.8)</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>51 (38.1)</td>
<td>293 (37.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td>0.255</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (6.7)</td>
<td>24 (3.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>125 (93.3)</td>
<td>749 (96.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>7 (5.3)</td>
<td>12 (1.6)</td>
<td>0.097</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0 (0.0)</td>
<td>8 (1.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>7 (4.5)</td>
<td>15 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>38.2 ±1.8</td>
<td>37.6 ±2.7</td>
<td>0.107</td>
</tr>
<tr>
<td><strong>Urgency of CS</strong></td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>Emergency</td>
<td>112 (83.6)</td>
<td>483 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Elective</td>
<td>22 (16.4)</td>
<td>290 (37.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Labour</strong></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Yes</td>
<td>94 (70.1)</td>
<td>713 (92.2)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>40 (29.9)</td>
<td>60 (7.8)</td>
<td></td>
</tr>
<tr>
<td><strong>PROM</strong></td>
<td></td>
<td></td>
<td>0.067</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (31.1)</td>
<td>84 (20.3)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>42 (68.9)</td>
<td>329 (79.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Chorioamnionitis</strong></td>
<td></td>
<td></td>
<td>0.126</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (4.9)</td>
<td>13 (1.7)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>127 (95.1)</td>
<td>760 (98.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Onset of labour</strong></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>40 (30.2)</td>
<td>75 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>94 (69.8)</td>
<td>698 (90.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Augmentation of labour</strong></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Yes</td>
<td>31 (23.1)</td>
<td>2 (0.3)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>103 (76.9)</td>
<td>772 (99.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Type of anaesthesia</strong></td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Spinal</td>
<td>116 (86.8)</td>
<td>760 (98.3)</td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>18 (13.2)</td>
<td>13 (1.7)</td>
<td></td>
</tr>
<tr>
<td>No. of days on admission (pre-delivery)</td>
<td>1.77±2.50</td>
<td>1.03±0.64</td>
<td>0.025*</td>
</tr>
<tr>
<td>No. of days on admission post delivery</td>
<td>3.36±1.03</td>
<td>3.33±0.88</td>
<td>0.809</td>
</tr>
<tr>
<td>Length of surgery (minutes)</td>
<td>63.3 ±17.1</td>
<td>73.2 ±21.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>Perineal shave</strong></td>
<td></td>
<td></td>
<td>0.616</td>
</tr>
<tr>
<td>Yes</td>
<td>121 (90.3)</td>
<td>696 (90.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13 (9.7)</td>
<td>77 (9.9)</td>
<td></td>
</tr>
<tr>
<td>Number of hours preceding surgery</td>
<td>11.4 ±14.6</td>
<td>1.5 ±3.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>Source of scrub water</strong></td>
<td></td>
<td></td>
<td>0.404</td>
</tr>
<tr>
<td>Running water</td>
<td>87 (64.9)</td>
<td>462 (59.8)</td>
<td></td>
</tr>
<tr>
<td>Stagnant water</td>
<td>47 (35.1)</td>
<td>311 (40.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant; prev.- Previous; SVD- spontaneous vaginal delivery; CS- Caesarean section; No.- Number; ANC-antenatal care; SVD- PROM- premature rapture of membranes; HIV- Human immunodeficiency virus.
4.3 Multivariate Model of Risk Factors for Post Caesarean Section Infections at KBTH.

Table 4.3 shows the multivariate logistic regression model developed to determine the significant predictors of post CS infections. Characteristics among the women with or without post CS infection compared in the multivariate analysis showed that, women are more likely to develop post CS infection when they are between the ages of 20 and 34 (p=0.003-0.038) especially those between 20 and 24, who had more than four (AOR=4.71) times risk of developing post CS infection. Also women who were married (p=0.020), had a history of alcohol consumption (p<0.001), had less than four (4) ANC visits (p=0.045), had emergency CS (p=0.029), were in labour before CS (p<0.001) or those who were prepared for surgery more than 8 hours before the surgery was performed (p=0.001) had a significant risk of developing post CS infection. The multivariate model also showed that the use of stagnant water as scrub water increased the risk of developing post CS infection by seven times (p=<0.001, AOR=7.07). On the other hand, surgery within 60 minutes (AOR=0.38) and administration of spinal anaesthesia (AOR=0.76) reduced the risk of developing post CS infection.
Table 4.3 Multivariate model of risk factors for post CS infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>AOR</th>
<th>95% C.I for AOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>0.168</td>
<td>16.56</td>
<td>2.56</td>
</tr>
<tr>
<td>20-24</td>
<td>0.003*</td>
<td>4.71</td>
<td>1.13</td>
</tr>
<tr>
<td>25-29</td>
<td>0.029*</td>
<td>1.27</td>
<td>0.40</td>
</tr>
<tr>
<td>30-34</td>
<td>0.038*</td>
<td>2.52</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>0.469</td>
<td>1.821</td>
<td>0.359</td>
</tr>
<tr>
<td>Normal weight</td>
<td>0.136</td>
<td>0.528</td>
<td>0.228</td>
</tr>
<tr>
<td>Overweight</td>
<td>0.292</td>
<td>1.446</td>
<td>0.728</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not married/previously married</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently married</td>
<td>0.020*</td>
<td>3.32</td>
<td>1.21</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife/unemployed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artisan</td>
<td>0.303</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>Trader/farmer</td>
<td>0.123</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>Professional</td>
<td>0.445</td>
<td>2.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Skilled worker</td>
<td>0.624</td>
<td>1.78</td>
<td>0.18</td>
</tr>
<tr>
<td>Student</td>
<td>0.988</td>
<td>0.98</td>
<td>0.06</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>&lt;0.001*</td>
<td>8.66</td>
<td>2.58</td>
</tr>
<tr>
<td>ANC &lt;4</td>
<td>0.045*</td>
<td>0.36</td>
<td>0.13</td>
</tr>
<tr>
<td>Emergency CS</td>
<td>0.029*</td>
<td>3.02</td>
<td>1.12</td>
</tr>
<tr>
<td>Labour before CS</td>
<td>&lt;0.001*</td>
<td>35.72</td>
<td>8.11</td>
</tr>
<tr>
<td>Spinal Anaesthesia</td>
<td>0.794</td>
<td>0.76</td>
<td>0.10</td>
</tr>
<tr>
<td>Length of surgery (&lt;60mins)</td>
<td>0.101</td>
<td>0.38</td>
<td>0.12</td>
</tr>
<tr>
<td>Hours preceding surgery (≥8hrs)</td>
<td>0.001*</td>
<td>9.18</td>
<td>2.63</td>
</tr>
<tr>
<td>Scrub water (Stagnant water)</td>
<td>&lt;0.001*</td>
<td>7.02</td>
<td>2.56</td>
</tr>
</tbody>
</table>

* Significant; BMI- Body mass index; ANC- Antenatal care; CS- Caesarean section
4.4 Aetiology of Post Caesarean Section infection at the KBTH

In this study, 119 isolates were cultured from the 134 wound swabs collected. Table 4.4 shows the frequency distribution of the organisms that are associated with causing post CS infections. The most common organism isolated was *S. aureus*. The bacteria isolated were mainly gram-positive cocci, gram-negative cocci and Enterobacteriaceae. Yeast from the *Candida* species were also isolated.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Frequency N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>23 (19.3)</td>
</tr>
<tr>
<td>CoNS</td>
<td>21 (17.6)</td>
</tr>
<tr>
<td><em>Escherichia. coli</em></td>
<td>17 (14.3)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>16 (13.4)</td>
</tr>
<tr>
<td><em>Proteus</em> species.</td>
<td>16 (13.4)</td>
</tr>
<tr>
<td><em>Klebsiella</em> species.</td>
<td>12 (10.1)</td>
</tr>
<tr>
<td><em>Citrobacter</em> species.</td>
<td>9 (7.6)</td>
</tr>
<tr>
<td><em>Candida</em> species</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td><em>Streptococcus</em> species.</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td><em>Actinetobacter</em> species.</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>
4.5 Resistance Pattern in *Staphylococcus* Isolates from Post CS infections

Figure 4.1 shows the resistance pattern among *S. aureus* and CoNS isolated. With the exception of gentamycin, these organisms showed resistance of more than 50% to all the antibiotics tested. Apart from *S. aureus*, which was almost 60% resistant to cefoxitin, both *S. aureus* and CoNS were most resistant to penicillins and cephalosporins.

*Figure 4.1* Resistance pattern among *Staphylococcus aureus* and CoNS isolates.
4.6 Resistance Patterns in Enterobacteriaceae isolated from Post Caesarean Section Infections

Figure 4.2 shows the resistance patterns among Enterobacteriaceae isolated. *E. coli* and *Citrobacter* species isolated were, at least, 30% resistant to all the antibiotics used except amikacin. *Klebsiella* and *Proteus* isolated were less than 20% resistant to amikacin and fluoroquinolones (ciprofloxacin and norfloxacin) but more than 50% resistant to the other antibiotics. The organisms were least resistant to amikacin and most resistant to piperacillin tazobactam.

Figure 4.2 Resistance patterns among Enterobacteriaceae.
4.7 Resistance Patterns in *Pseudomonas aeruginosa* Isolated from Post CS Infections

Figure 4.3 shows resistance patterns in *P. aeruginosa* isolated. Apart from the fluoroquinolones, *P. aeruginosa* isolated were more than 20% resistant to all the antibiotics. It was most resistant to ceftazidime.

![Figure 4.3 Resistance patterns among *Pseudomonas aeruginosa*](image-url)
4.8 Prevalence of Methicillin Resistant *Staphylococcus* Species and Extended spectrum Beta-Lactamase Producing Enterobacteriaceae

Table 4.5 shows the prevalence of mecA mediated methicillin resistance among *Staphylococcus* isolates and ESBL producing strains of *E. coli* and *Klebsiella* isolated. Phenotypic and PCR methods indicated that, some of the *S. aureus* and CoNS isolated were MRSA, methicillin resistant CoNS and ESBL. In the case of CoNS, *E. coli* and *Klebsiella* species, all the isolates, which were phenotypically found to be positive, were confirmed by PCR. However, one of the MRSA, which was positive phenotypically, did not possess the *mecA* gene.

**Table 4.5** Prevalence of mecA mediated methicillin resistance among *Staphylococcus* isolates and ESBL producing strains of *E. coli* and *Klebsiella* isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Total No.</th>
<th>Screening method</th>
<th>Phenotypic N (%)</th>
<th>PCR N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em>#</td>
<td>23</td>
<td></td>
<td>13 (56.5)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>CoNS#</td>
<td>21</td>
<td></td>
<td>18 (85.7)</td>
<td>18 (85.7)</td>
</tr>
<tr>
<td><em>E. coli</em>*</td>
<td>17</td>
<td></td>
<td>6 (35.3)</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td><em>Klebsiella spp</em></td>
<td>12</td>
<td></td>
<td>4 (33.3)</td>
<td>4 (33.3)</td>
</tr>
</tbody>
</table>

# = ESBL producing strains; * = MecA mediated methicillin resistant genes.
4.8.1 Distribution of ESBL variants among Enterobacteriaceae causing post CS infection

Table 4.6 shows the distribution of ESBL variants between *E. coli* and *Klebsiella* isolated.

The ESBL positive *E. coli* strains were found to have either *bla*$_{CTX}$-*M*, *bla*$_{TEM}$ or both *bla*$_{CTX}$-*M* and *bla*$_{TEM}$ genes. More than 80% of the *E. coli* possessed *bla*$_{CTX}$-*M* gene. However, ESBL producing *Klebsiella* isolates had either *bla*$_{TEM}$, *bla*$_{SHV}$ or both *bla*$_{TEM}$ and *bla*$_{SHV}$ genes. *E. coli* had no *bla*$_{SHV}$ genes and *Klebsiella* had no *bla*$_{CTX}$-*M* genes. The *bla*$_{TEM}$ gene was found to cut across the two groups of ESBL producing organisms.

<p>| Table 4.6 Distribution of ESBL gene variants among Enterobacteriaceae isolated |
|-------------------------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|</p>
<table>
<thead>
<tr>
<th>Isolates</th>
<th>Total No.</th>
<th><em>bla</em>$_{CTX}$-<em>M</em></th>
<th><em>bla</em>$_{TEM}$</th>
<th><em>bla</em>$_{SHV}$</th>
<th><em>bla</em>$<em>{CTX}$-*M$ &amp; <em>bla</em>$</em>{TEM}$</th>
<th><em>bla</em>$<em>{TEM}$ &amp; <em>bla</em>$</em>{SHV}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>6</td>
<td>5 (83.3)</td>
<td>3 (50)</td>
<td>-</td>
<td>2 (33.3)Q</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella</em> species</td>
<td>4</td>
<td>-</td>
<td>3 (75)</td>
<td>3 (75)</td>
<td>-</td>
<td>2 (50)</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of Post Caesarean Section Infections at the KBTH

The prevalence of post CS infections varies with different patient populations. The prevalence of above 10% reported by this current study is higher than what has been found in other studies (Ezechi et al., 2009; Jido & Garba, 2012; Nsiah–Afriyie, 2017). However, it was within the range reported by some studies. Ward et al. (2008) reported post CS infections to be up to 18% in the UK. They reported such high prevalence because they included post discharge surveillance. The current study conducted, did not specifically consider post discharge surveillance but patients who reported to the facility after discharge were included. Even though the prevalence reported was lower than 18%, it can be inferred that the prevalence recorded could be higher if post discharge infections were followed up. As the study conducted by Griffiths et al (2005) all the subjects from the current study were given antibiotic prophylaxis (Amoxicillin and clavulanic acid, metronidazole and gentamicin). In that study, the prevalence was 9.9% Griffiths et al. (2005). Therefore, such high rates of post CS infections after administration of prophylaxis is an indication that, the antibiotics given at the KBTH for post CS infection prophylaxis should be reviewed.

5.2 Risk factors for Post Caesarean Section Infections at the KBTH

Studies done by other researchers found age (especially younger age), obesity, diabetes, chorioamnionitis, PROM, emergency CS and longer operation time to be risk factors for post CS infections (Tran et al., 2000; Killian et al., 2001; Johnson et al., 2006; Olsen et al., 2008). Conversely, in the current study chorioamnionitis, diabetes and PROM had no association with the development of SSI among women who had CS. This study also established that, longer operating durations above 60 minutes was a significant risk of developing SSI. This is
may be because longer operating durations could increase the risk of contaminating the incision site by organisms in the environment. Contrary to other studies, obesity was not an independent risk of developing post CS infection in the multivariate studies even though BMI was significant in the univariate analysis (Tran et al., 2000; Schneid-Kofman et al., 2005).

Inconsistent with this study, Tran et al. (2011) in a study done in Malaysia reported spinal anaesthesia to be associated with a higher risk of developing post CS infection. Findings from Tsia et al. (2011) however, found general anaesthesia to be a risk of post CS infection (Tran et al., 2000; Tsai et al., 2011).

The absence of correlation between post CS infection and previous form of delivery found in this study agrees with Jasim et al. (2017). However, unlike this study Jasim and his colleagues found no correlation between emergency CS and post CS infection. Patients who go through emergency CS usually do not get enough preparation time, which could increase the risk of contamination. This study has also found that patients who are prepared eight or more hours before the surgery was performed had a significant risk of developing post CS infections. Another risk factor found in this study was the use of stagnant water (water stored in barrels) as scrub water before the surgery. The use of stagnant water compared to the use of running tap water increased the risk of post CS infection by seven (7) times. Water stored in barrels left for days can easily be contaminated hence when used for washing hands before performing surgery, increases the chances of contracting post CS infection.
5.3 Etiological Agents Responsible for Post Caesarean Section infections at the KBTH

The varying aetiological agents observed was not far from the expected because other studies also reported similar organisms (Khairy et al., 2011; Dhar et al., 2014). As in other studies, *S. aureus* was the most frequently isolated organism. It is the most common organism responsible for not only post CS infections but also wound infections in general observed across the world (Giacometti et al., 2000; Bessa et al., 2015; Rai et al., 2017). This can be attributed to the fact that *S. aureus* is a nasal carriage organism (Ruffing et al., 2017), which can disseminated by contact (Obadia et al., 2015).

Additionally, *S. aureus* and other gram-positive cocci are normal flora of the skin (Chiller et al., 2001). This makes incisions on the skin prone to infections by these organisms and it could account for the frequent isolation of CoNS and *Streptococci* species. *Escherichia coli* was also prevalent probably because of the space proximity of the incision with the perineum. Other organisms from exogenous sources like *P. aeruginosa* and *Actinetobacter* species were properly found because of contamination by health professionals who directly handled the incision, surgical instruments and materials and the theatre environment (Kirby & Mazuski, 2009).

5.4 Antimicrobial Susceptibility of Etiological Agents Isolated from Post Caesarean Section Infections

The generally high level of resistance patterns observed across both gram-negative is gram-positive organisms is disturbing. Among gram-negative organisms, amikacin showed the lowest level of resistance probably because it is only available in injectable form hence, not rampanty used as the other antibiotics available in oral form. Resistance to third generation
cephalosporin could be due to the fact that, these antibiotics are usually used in the treatment of syndromic infections and they are easily accessible (MOH, 2010). Resistance was exhibited towards fluoroquinolones compared to other classes of antibiotics was low. Nevertheless, the potential rise in fluoroquinolone resistance in Ghana cannot be ignored because fluoroquinolones are the first line treatment for blood stream infections and urinary tract infection in Ghana (MOH, 2010). In the hospital setting especially these drugs are likely to be overused because the options provided by the Standard Treatment Guidelines (STG) are limited. Considering the presence of ESBL strains, it is not surprising to find such patterns of resistance among cephalosporins and fluoroquinolones ESBLs have been established to be associated with cephalosporin and fluoroquinolone resistance in Ghana (Obeng-Nkrumah et al., 2013; Wireko et al., 2017).

5.5 Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant Coagulase Negative *Staphylococcus* Isolated from Post Caesarean Section Infections

The prevalence of MRSA found by this study is significantly higher than what was reported by Egyir *et al.* (2014) in Ghana. In their research, they recorded as low as 3% prevalence of MRSA (Egyir et al., 2014). This sharp difference could be evidence of a rise in the prevalence of MRSA especially among hospitalized patients.

The higher prevalence of methicillin resistant CoNS when compared to MRSA observed is similar to that observed in other countries. A study conducted in 12 countries in Europe reported an average prevalence of 71.5% for Methicillin resistant CoNS and 29.1% for MRSA (Sader *et al.*, 2007).
5.6 Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae Isolated from Post Caesarean Section Infections

This study found a high prevalence of ESBL among *E. coli* and *Klebsiella* isolates obtained from wound swabs in women who developed post CS infections in the KBTH. The prevalence of ESBLs among Enterobacteriaceae reported in this current study agrees with other studies (Feglo, 2011; Dhar *et al.*, 2014). Feglo (2011) reported ESBL prevalence of 57.8%, which is higher than what was found by this current study. (Feglo, 2011).

Studies done by other researchers also reported the presence of more than one ESBL genotype in one isolate (Yamasaki *et al.*, 2003; Munday *et al.*, 2004; Seyedjavadi *et al.*, 2016). The distribution of ESBL variants among the Enterobacteriaceae isolated in this current study was similar to what was found by Paterson and Bonomo (2005). They found that *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes were the genes associated with ESBL production among Enterobacteriaceae with *bla*<sub>CTX-M</sub> being the most predominant. However, contrary to the current study, they reported *bla*<sub>SHV</sub> genes in *E. coli*. (Paterson & Bonomo, 2005).
CHAPTER SIX

6.0 CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of post CS infections in the KBTH found by this study was more than 10%. Risk factors for post CS infections included patient sociodemographic history, obstetric history and institutional factors.

A variety of aetiological agents was found with S. aureus being the most prevalent. Extended spectrum beta-lactamase producing organisms, MRSA and methicillin resistant CoNS were also found to be associated with post CS infections. The organisms exhibited the most resistance to penicillins and cephalosporins.

6.2 Limitations

The study did not follow up on the patients who had been discharged for the true picture of the burden of post CS infections to be determined.

The samples used for the study were not subjected to anaerobic conditions to identify if any anaerobic organisms are associated with post CS infections.
6.3 Recommendations

Firstly, this study showed that there is a high prevalence of post CS infections at the KBTH. To control this rate, regular surveillance should be carried out. In addition, this study should be repeated in other facilities in the country to determine the true picture of post CS infections in the country.

In this study, MRSA, methicillin resistant CoNS and ESBLs were found among the isolates. However, sequencing was not done on these isolates to determine the strain types. It is recommended that these isolates as well as isolates subsequently should be sequenced.

Multidrug resistant patterns of MRSA, methicillin resistant CoNS and ESBLs have serious implications for treatment. Therefore, routine testing and regular monitoring of antibiotics to which the organisms are resistant should be incorporated in surveillance systems of the country.

It is also recommended that, to control resistance patterns, as much as possible, antimicrobial susceptibility results should be consulted before treatment for infections are begun.
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APPENDIX I

PARTICIPANTS CONSENT FORM

CONSENT TO PARTICIPATE IN A STUDY ON SURGICAL SITE INFECTIONS FOLLOWING CAESAREAN SECTIONS AT THE KORLE-BU TEACHING HOSPITAL.

Investigator:

Mary Abena Attah

Department of Medical Microbiology

School of Biomedical and Allied Health Sciences

Korle-Bu Teaching, Accra, Ghana

Email: absafoa@yahoo.co.uk

Phone number: +233543033317

Information: (to be read or translated to the participant in her language of preference)

Dear participant,

The researcher is an M.Phil. Microbiology student of the Medical Microbiology Department of the School of Biomedical and Allied Health Sciences and is undertaking a research entitled: “Post Caesarean Section Infections at the Korle-Bu teaching Hospital” This Consent Form contains information about the research which you are required to know before you accept to participate in the study. You will need to read through the information thoroughly or have it read out to you in a language you are comfortable with. You will then append your signature or thumb-print in front of a witness. If you are unfamiliar with or do not understand any part of the text, kindly seek clarification from whoever is administering the questionnaire to you. After signing or thumb-printing, a copy of this form will be given to you, kindly keep it safe for future reference.
PURPOSE OF THE STUDY

This purpose of this study is to determine the prevalence of surgical site infections following caesarean sections and determine the factors that predispose the patient to these infections. Surgical site infections are infections, which occur after surgery in the part of the body where the surgery took place. These infections may be superficial involving the skin only or may involve deeper structures beneath the skin known as subcutaneous tissues and organ spaces. These infections pose a major challenge to the new mothers and their recovery from surgery. They predispose them to longer hospital stay, increased hospital costs, interfere with breastfeeding and bonding with their new babies and are major causes of postpartum depression.

Surgical site infections place huge financial burdens on the patient, their families, the NHIS, health Institutions and Ghana as a whole. This study will attempt to determine the incidence of this surgical complication following Caesarean sections in the KBTH, selected institutional risk factors and determine the cost of these infections on the patient and the hospital. There is very little data on this subject in KBTH and Ghana.

GENERAL INFORMATION ON THE STUDY

To participate in this study, you will have to be scheduled for a caesarean section, electively or as an emergency at the maternity unit of the KBTH. You should also have consented to be included in the study. We will collect some basic demographic, obstetric and relevant personal information about you using a one-time maternal data abstraction form. This will take about 15 minutes. There are no costs, immediate benefits or risks posed to you as a participant. Some sensitive information may be required from you and all steps will be taken to employ sensitive interviewing techniques to maintain privacy and confidentiality. The researcher only requires a few minutes of your time and candid answers to the questions posed. Your participation in this study will benefit other pregnant women undergoing
caesarean section.

CONFIDENTIALITY

The researcher and his team guarantee that all information obtained from you shall be held in the strict confidence to the best of our ability. Your identity will be anonymous and no direct reference will be made to you. If you have any questions or reservations at any point during the study, kindly direct them to the researcher.

WITHDRAWAL FROM THE STUDY

This study is strictly voluntary. If you decide not to participate in the study or at any point decide to opt out of the study, you are free to do so with no consequences. Your decision will not affect the care you receive in the hospital.

This study has been reviewed by the Ethical and Protocol Review Board of the College of Health Sciences, University of Ghana. Any ethical questions or reservations concerning your rights as a participant in this study can be directed to:

The Chairman, EPRC, CHS, University of Ghana

Email – eprc@chs.edu.gh
Phone number – 0244061270

Any other general questions about the study can be directed to the Investigator,

Mary Abena Attah

Medical Microbiology Department
VOLUNTEER AGREEMENT

INFORMED CONSENT

The above document describing the procedures for the research titled “Post Caesarean Section Infections at the Korle-Bu teaching Hospital” has been read and explained to me in a language I understand. I have asked all my questions concerning my participation in the study and they have been answered satisfactorily. I hereby consent voluntarily to participate in the study.

Date                                                                             Signature or Thumbprint of volunteer, Parent or guardian.

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Date                                                                             Signature of interviewer / Person who obtained consent

For participants who cannot read and write or incapacitated:

I was present while the content of this consent form was read to the volunteer. All questions
were answered and the volunteer has agreed to take part in the research.

<table>
<thead>
<tr>
<th>Date</th>
<th>Signature or Thumbprint of witness</th>
</tr>
</thead>
</table>

University of Ghana http://ugspace.ug.edu.gh
APPENDIX II

ADOLESCENT PARTICIPATION ASSENT FORM:

My name is Dr. Chibuikem Nwabugwu Onuzo and I am from the Department of Obstetrics and Gynaecology, KBTH. I am undertaking a research into “Post Caesarean Section Infections at the Korle-Bu teaching Hospital”. A research is usually conducted to learn more about a medical condition and how to improve it.

I am asking you to take part in this research study because I am trying to learn more about surgical site infections following a caesarean section, how common it is in Korle-Bu and the risk factors to the infections. If you decide that you want to be part of this study, some information concerning your pregnancy and delivery will be collected from your records. You will also be asked a few personal questions and about your family. These questions will take about 15 minutes.

There are some things about this study you should know. There will be no procedures conducted on you, you will not have to pay to participate in the study, you will not be hurt in any way during the study and we are the only people who will know what you say or do. You may not benefit directly from the research but another mother may do in future. If you are not happy answering some of the questions we ask you, kindly let us know and we will skip them.

You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that’s okay too. No one will be angry with you if you do not want to participate or decide to stop at any time. Your information will be kept confidential, this report will not include your name or that you were in the study, no one will be able to know how you responded to the questions and your information will be anonymous.

If you want to know more about the study, it is okay to ask questions. You can call this number at any time, 0244684309 or talk to me the next time you see me.

Your parents or guardian know about the study too and have given their okay to your
participation but please talk to them before you decide to participate in the study. Even if they say yes, you can still decide not to participate.

Any ethical questions or reservation about the study can be directed to

The Chairman, EPRC, CHS, University of Ghana

Email – eprc@chs.edu.gh

Phone number – 0244061270

VOLUNTARY AGREEMENT

By making a mark or thumb printing below, it means that you understand and know the issues concerning the research study. If you do not want to participate in the study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research titled “Post Caesarean Section Infections at the Korle- Bu teaching Hospital” has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate.

Adolescent’s name ________________________________

Researcher’s name ______________________________

Adolescent’s Mark/Thumbprint  Researcher’s Signature

........................................                                           ..........................................

Date ___________________________________
APPENDIX III

STRUCTURED QUESTIONNAIRE

TITLE

“POST CAESAREAN SECTION INFECTIONS AT THE KORLE-BU TEACHING HOSPITAL”

SECTION 1:

SOCIO-DEMOGRAPHICS

Client identification number: ........................................

1. Age…

2. Marital Status:
   a. Married - 1       Single - 2
   b. Co-habiting – 3   Divorced/Separated – 4       Other -5 (please specify)

3. Highest level of education:
   a. No education - 1       Primary - 2
   b. Junior high School – 3   Senior high School - 4
   c. Tertiary and above – 5

4. Occupation: .................................................................

5. Do you drink alcohol?     YES – 1     NO - 2
6. Do you smoke tobacco?        YES – 1      NO - 2

SECTION 2:

OBSTETRIC DATA

7. Gravidity: ..............

8. Parity: .................

9. Previous mode of delivery  Vaginal – 1       Caesarean Section - 2
10. Number of previous CS ..........................

11. Antenatal Care:  Yes – 1        None - 2

12. Number of visits .................

13. Level of Antenatal Care:         TBA – 1   Maternity Home – 2   CHPS compound
                                          – 3          Clinic - 4     Hospital - 5

14. Number of antenatal visits: ...........

SECTION 3:
MATERNAL RECORDS ABSTRACTION FORM

15. Weight at booking: .......... kg

16. Height at booking .......... Meters

17. BMI at booking .............. kg/m²

18. Gestational Age at booking: ............ weeks

19. Urine proteins on day of delivery......................

20. Is the patient diabetic:  Yes (1)    No (2)

21. Pre-Gestational DM:  Yes (1)     No (2)

22. Gestational DM:    Yes (1)    No (2)

23. RBS on day of delivery: ........ mmol/L

24. Maternal Infections

25. HIV  Reactive - 1          Non-reactive – 2

26. Syphilis (VDRL)          Reactive - 1          Non-reactive – 2

27. Hepatitis BsAg          Reactive - 1          Non-reactive – 2
DELIVERY RECORDS

28. Gestational age at delivery: ……………… weeks
29. Urgency of CS: Elective (1) Emergency (2)
30. Indication for CS: ……………………………………………………
31. Did labour precede the CS: Yes (1) No (2)
32. Prelabour Rupture of Membranes Yes – 1 No – 2
33. Duration ……………………
34. Diagnosed chorioamnionitis: Yes (1) No (2)
35. Number of vaginal examinations: …………………………………
36. Onset of labour Spontaneous labour - 1 Induced labour – 2
37. Indication for Induction: ……………………………………………
38. Need for labour augmentation: YES – 1 NO – 2
39. Duration of labour preceding CS: ……………………………….. hours
40. Duration of stay on the labour ward: ……………………………… hours
41. Type of anaesthesia: Spinal – 1 General – 2
42. Number of days pre delivery on admission ……………… days
43. Number of days post-delivery on admission …………… Days
44. Level of expertise of Surgeon:
   Consultant (1) Senior Resident (2) Junior Resident (3) Medical Officer (4)
   House Officer (5)
45. Length of surgery: …………… minutes
46. Perineal shave: Yes (1) No (2)
47. Number of hours preceding surgery: …………… Hours
48. Source of scrub water: Running water (1) Stagnant water (2)
49. Time of surgery: 8am - 5pm (1) 5pm – 8am (2)
50. Type of incision: Pfannenstiel (1) Midline (2)
51. Antibiotic prophylaxis: Yes (1) No (2)
   (ii)Names: …………………………………………………
52. Number of minutes/hours: ……………
53. Surgical complication: Yes (1) No (2)
   (ii)Type: ………………………………………………………
Interviewer: Name: ......................................

Signature: ...........................................

Date: .............................................
APPENDIX IV

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

CHS/EPRC/JULY/2018

July 03, 2018

Ref. No.: .....................................................

Mary Abena Attah
Department of Medical Microbiology
School of Biomedical and Applied Health Sciences
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: CHS-Et/M.10 – P2.6/2017-2018

The College of Health Sciences Ethical and Protocol Review Committee on June 28, 2018 reviewed and unanimously approved your research proposal.

Title of Protocol: "Post Cesarean section infections in Korle Bu Teaching Hospital"

Principal Investigator: Mary Abena Attah

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till July 03, 2019.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....................................................

Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

Cc: Provost, CHS
Dean, SBAHS
Head, Medical Microbiology