Vaginal Colonization Rate of Methicillin Resistant *Staphylococcus aureus* among Pregnant Women Attending Antenatal Clinic: Ayder Teaching and Referral Hospital, Mekelle, Tigray, Ethiopia.

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JUNE, 2012
ADDIS ABABA UNIVERSITY

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A thesis Submitted to the School of Graduate Studies, Addis Ababa University: in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Medical Microbiology

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JUNE,2012

ACKNOWLEDGEMENTS

I strongly acknowledge my advisors Dr. Daniel Asrat (MD, M.Sc, PhD) and Dr. Yimtubezinash W/Amanuel (MD, M.Sc, PhD) for their unreserved advice and careful comments throughout my thesis work. Without their help the accomplishment of this thesis would have been impossible.

I am grateful to Dr. Angesom Kebede (MD, Gynecologist) head of the department of Gynecology and Obstetrics at Mekelle University, Ethiopia; my co-supervisor and I gratefully acknowledge him for providing unreserved supervision and encouragement during the work of this study.

Special thanks due to staff of Gynecology and ANC of Ayder Teaching and Referral Hospital; Dr. Teshale Wubete, S/r Sara, S/r Haregewein, and S/r Fethin for their unreserved and invaluable contribution during sample collection and processings.

I would also like to acknowledge all the staff members of the Department of Microbiology, Immunology and Parasitology of Biomedical science institution of Mekelle University.

I would like to extend my acknowledgement to members of Microbiology, Immunology and Parasitology Department and friends who in one way or another contributed for the successful accomplishment of this work.

Finally, I would like to Acknowledge Addis Ababa University for giving me the chance to join this program and finance source.

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ABBREVIATIONS

CA-MRSA	Community Associated Methicillin *Staphylococcus aureus*
CDC	Centers for Disease Control and Prevention
Clf	Clumping Factor
ClfA	Clumping Factor A
ClfB	Clumping Factor B
CLSI	Clinical and Laboratory Standards Institute
Cnal	Collagen Binding Protein
CSA	Central Statistical Agency
FnBP	Fibronectin Binding Protein
FnBPA	Fibronectin Binding Protein A
FnBPB	Fibronectin Binding Protein B
GBS	Group B *Streptococcus*
GSIA	Glycopeptides Intermediate Susceptible *Staphylococcus aureus*
HA-MRSA	Healthcare Associated Methicillin *Staphylococcus aureus*
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSCRAMM	Microbial Surface Components Recognizing Adhesive Matrix Molecule
MSSA	Methicillin Sensitive *Staphylococcus aureus*
NCCLS	National Committee for Clinical Laboratory Standards
NNIS	National Nosocomial Infection Surveillance System
SCC	*Staphylococcu*s Cassette Chromosomes
SSSS	*Staphylococcal* Scalded Skin Syndrome
TNF-R1	Tumor Necrosis Factor- Receptor-1
TSS	Toxic Shock Syndrome
TSST-1	Toxic shock Syndrome Toxin-1

ABSTRACT

Background: Early epidemiological studies indicated that 5% of women were colonized with *Staphylococcus aureus* (*S. aureus*) in their genital tract. Postpartum women had the highest colonization rates. Studies, mostly from United States have reported that vaginal colonization rates for *S. aureus* in pregnant women range from 14% to 17.1% and it is the major cause of surgical site infection. It was also indicated that between 25% and 50% of women undergone cesarean section develop post surgical site infections because of *S. aureus*.

Objective: The aim of this study was to determine the prevalence of genital tract colonization of *S. aureus* and MRSA among pregnant women at Ayder teaching and referral hospital of Mekelle University.

Methods: A cross sectional study was carried out from December, 2011 and February, 2012 using non probable convenient sampling technique for screening vaginal cultures for *S. aureus* obtained from 190 pregnant women (at and greater than 24 weeks of gestation) and were processed for identification of *S. aureus* including methicillin- resistant strains.

Results: From 190 pregnant women culture result was available from 184 pregnant women with culture data in the study and of these 43 (23.4%) were colonized with *S. aureus*. Out of the 43 isolates, 12 (27.9%) were MRSA positive. The antimicrobial susceptibility pattern for 43 *S. aureus* of the isolates showed 100 % resistant to ampicillin , amoxycillin , and penicillin followed by high resistance to tetracycline (83.7%), erythromycin (41.9%), oxacillin (27.9%), ciprofloxacilin (18.6%), gentamicin (4.6%), and to vancomycin (2.3%). Multi-resistance to two or more antimicrobial agents was observed in 100% of all tested *S. aureus*.

Conclusion and recommendation: This study provided data on the vaginal carriage rate of MRSA and initial information on the antibacterial resistance pattern in *S. aureus* obtained from randomly selected pregnant women. In our results, high level of MRSA and multi drug resistance was observed. Therefore, we recommend that additional studies with more epidemiologic tools are needed to further asses the role of *S. aureus and* MRSA colonization in pregnant women in this study area.

Key Words: Prevalence, *Staphylococcus aureus*, pregnant women, MRSA, Ethiopia

CHAPTER I- INTRODUCTION

1.1. General Introduction

Staphylococcus aureus (*S. aureus*) is a significant human pathogen accountable for nosocomial and community acquired infections, which also behaves as commensal flora in healthy persons – mainly colonizing the anterior nares (Peacock *et al*., 2001). *S. aureus* is an encountered by obstetricians, gynecologists, and neonatologists. All strains of *S. aureus* were sensitive to penicillin when it was introduced in the 1940s. Over a short period; the majority strains isolated from hospitalized patients were determined to be resistant. Methicillin, introduced in the early 1959, is one of many variants of the original penicillin molecule (Chambers, 2001). Resistance to Methicillin by strains of *S. aureus* developed quickly and for decades, Methicillin Resistant *Staphylococcus aureus* (MRSA) has been the most commonly recognized multidrug-resistant pathogen in Europe, the Americas, Asia, the Middle East, and Africa (Groundmann *et al*.,2006).

Increasing incidence of MRSA is a well-documented healthcare and community phenomenon of great concern to medical, public health, and lay communities around the world. In the early 1990s, MRSA was reported to account for 20–25% of *Staphylococcus aureus* isolates in hospitalized patients in the United States. By the last decade, many hospitals experienced MRSA percentages in the range of 50 - 70% of total *Staphylococcus aureus* isolates from clinical cultures (Siegel *et al*., 2006). Similarly, National Nosocomial Infections Surveillance System (NNIS) data analysis for 1992 to 2003 showed that the percentage of *S. aureus* isolates that were MRSA increased from 35.9% in 1992 to 64.4% in 2003 in USA participating the adult and pediatric clinic (Klevens *et al*., 2006).

MRSA has a record of being recurrently linked with healthcare, and conventional insight has grouped MRSA as a hospital problem until the late 1990s. However, Canadian MRSA surveillance system as showed that 5 up to 7% MRSA infections have been increasingly isolated from patients in the community who do not have the distinctive risk factors for MRSA infection including a history of prolonged broad-spectrum antibiotic use, intensive care unit admission, surgery, or exposure to other MRSA-infected patients (Conly *et al*., 2003).

In recent decades, MRSA strains, especially those associated to the community called community associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA)] have become important issues in public health, since they are a reason of increased morbidity and mortality (Zetola *et al*., 2005). These CA-MRSA strains cause serious skin and soft tissue infections, necrotizing pneumonia, and sepsis in healthy children, teens, and, more recently, in neonates (Huang *et al*., 2009). The rising number of infections in neonates caused by CA-MRSA emphasizes the need to recognize the likely environmentally or maternally derived sources of infection (Huang *et al*., 2009; Reusch *et al*., 2008).

In pregnant women, *S. aureus* also causes a health risk; because it is the major cause of infection of the surgical site, causing between 25% and 50% of infections of the post cesarean surgical site, representing a major cause of morbidity and a cause of puerperal mastitis (Laibl *et al*., 2005). Early epidemiological studies showed that 5% of women were colonized with *S. aureus* in their genital tract and postpartum women had the highest colonization rates; in addition, vaginal-rectal carriage of *S. aureus* has been found associated with development of postpartum fever (Chen *et al*., 2007).

Although risk factors related to colonization with MRSA strains during pregnancy have not been fully characterized, associations with race, parity, type of birth, and colonization with group B *streptococci* have been suggested (Chen *et al*., 2007). It is recognized that incidence of CA-MRSA infections differs between different communities and populations, and apparently, pregnant women are more vulnerable and have risk factors that predispose them to developing these infections (Laibl *et al*., 2005; Stumpf *et al*., 2008). Nevertheless, there is a scarcity of epidemiologic reports about MRSA infections present in pregnant women (Laibl *et al*., 2005).

In Ethiopia, a number of studies have been conducted to determine the prevalence of different bacterial profiles including MRSA in the overall population using different study participants and criteria's (Genet *et al*., 2012; Assefa *et al*., 2008). A study was conducted to determine the antimicrobial resistance pattern of common bacteria isolates during January 2003 to July 2004 in Jimma Hospital. A total of 291 Gram-positive and Gram-negative bacterial strains were isolated and examined from different clinical samples and body sites. The result has indicated that the prevalence of MRSA in this study were 8.3% (Gebre-Selassie, 2007).

A cross--sectional study was also done on 162 *S. aureus* and 59 coagulase-negative *staphylococci* from 151 inpatients and 70 outpatients at Bahr Dar Felege Hiwot Referral Hospital from April to June, 2006. The overall prevalence of MRSA was found to be 59.7% which was high and this high rate of MRSA with its multi-drug resistance will pose a big challenge in therapy of MRSA (Abera *et al*., 2008).

In fact as stated earlier, there are several studies which have been conducted in hospitals of major cities in Ethiopia. The aim of these studies was to determine the antimicrobial susceptibility of clinical bacterial isolates. However, there are no studies specifically conducted on female genital tract colonization of *S. aureus* and MRSA among pregnant women. As a result, this study was aimed to survey and determine the antimicrobial susceptibility of *S. aureus* vaginal isolates from pregnant women to mainly oxacillin and selected antimicrobial drugs that are commonly used in Mekelle town Ethiopia.

1.2. Literature Review

1.2.1. Historical Background

For the first time in 1880, Ogston described *staphylococcal* disease and its role in sepsis and abscess formation. Although Ogston was not the first to examine pus microscopically and describe micrococcus (from the Greek kokkos, meaning berry), those in chains had already been designated *Streptococci* by Billroth in1874. In 1882, Ogston named the clustered micrococcus "*staphylococci*," from the Greek "*staphyle*", meaning bunch of grapes. In 1884, a German surgeon, Anton J. Rosenbach (1842-1923), isolated two strains of *staphylococci*. He named for the pigmented appearance of their colonies: *Staphylococcus aureus*, from the Latin aurum for gold, and *Staphylococcus albus* (now called epidermidis), for white (Ogston, 1881).

1.2.2. Microbial Characteristics of the Genus *Staphylococcus*

The genus *Staphylococcus* contains more than 32 species and 15 subspecies which are widespread in nature (Evangelista *et al*., 2002). Medically, the first three of them are significant human pathogens: *Staphylococcus aureus*, which causes various pyogenic infections like endocarditis, osteomyelitis; skin and soft tissue infections; toxin-mediated diseases such as food poisoning, toxic shock syndrome and the scalded skin syndrome; *Staphylococcus epidermidis*, which causes infections associated with foreign bodies, such as catheters and prosthetic devices and also a common member of the skin flora; and *Staphylococcus saprophyticus*, which causes urinary tract infections; *Staphylococcus hominis and Staphylococcus haemolyticus* also less important human pathogens (Ryan , 2004).

Staphylococcus aureus is a member of the Micrococcaceae family and a major pathogen of increasing importance due to the rise in antibiotic resistance (Lowy, 1998). It is distinct from the Coagulaze negative *Staphulococcus* (e.g. *S. epidermidis*), and more virulent despite their phylogenic similarities. The species named *aureus*, refers to the fact that colonies (often) have a golden colour when grown on solid media, whilst coagulase negative *staphylococcus* (CoNS) form pale, translucent, white colonies (Broks, 2007).

1.2.3. Virulence Factors

S. aureus produces a number of virulence factors, most of which act in a synergistic and coordinated fashion. Some appear to be specifically associated with certain severe infections and are produced by MRSA clones distributed worldwide (Beigi *et al*., 2009). Super antigenic exotoxins appear to be major virulence factors in hospital MRSA clones (HA-MRSA), and *staphylococcal* enterotoxin A (SEA) may be involved in the physiopathology of septic shock (Tristan *et al*., 2005). Panton Valentine Leucocidin (PVL) has emerged as a major virulence factor in community-acquired *Staphylococcus aureus* (CA-MRSA) infections. In particular, the leukotoxic action of PVL is responsible for the high mortality rate associated with necrotizing pneumonia. CA-MRSA can also harbor the toxic shock syndrome toxin 1 (TSST-1) and rarely the exfoliative toxin (Tristan *et al*., 2005). Diagrammatic representation Virulence factors of *Staphylococcus aureus* is illustrated in Figure 1.1.

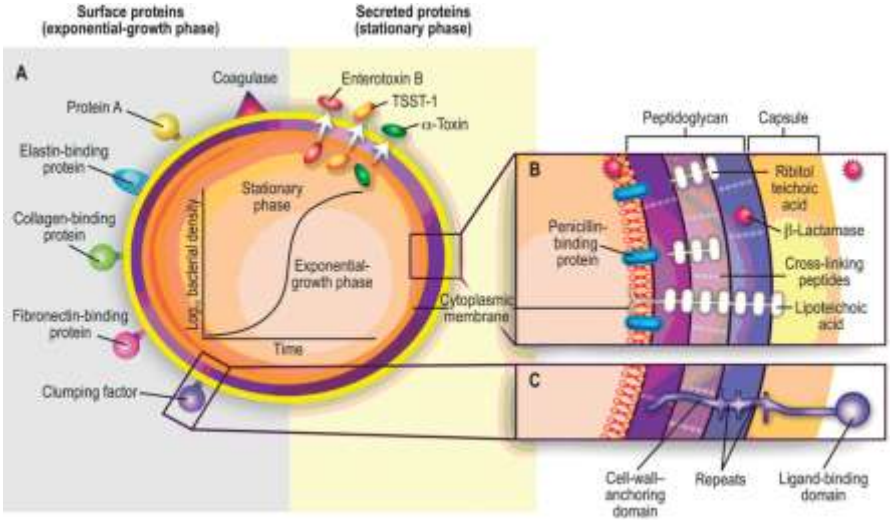

Figure1.1: Virulence factors of *Staphylococcus aureus*, with structural and secreted products both playing roles as virulence factors. (Adapted from: Lowy, 1998)

There are also some of the several toxins that have the potential to manipulate components of the immune response. These include leukotoxins that specifically affect white blood cells (WBC); (PVL and leukocidin D-E [LukD-E]); toxins that function as super antigens can manipulate the immune system by hyper stimulating the release of cytokines (SE-A, SE-B,

and TSST); and hemolysins that are active to lyses red blood cells and white blood cells (WBCs) as well as other cell types (Arbuthnott *et al*., 1990).

1.2.4. Pathogenesis

Staphylococcus aureus is a versatile pathogen capable of causing a wide range of human diseases. However, the role of different virulence factors in the development of *staphylococcal* infections remains incompletely understood. Some clonal types are well outfitted to cause disease across the globe, whereas others are simplistic at causing disease among community members. Although MRSA strains are not necessarily more virulent than methicillin-sensitive *S. aureus* strains, some MRSA strains contain factors or genetic backgrounds that may increase their virulence or may enable them to cause particular clinical syndromes (Rachel and Franklin, 2008).

The armamentarium of virulence factors of *S. aureus* is wide-ranging, with both structural and secreted products playing a role in the pathogenesis of infection (figure 1.1). Two important features of *staphylococci* are that a virulence factor may have several functions in pathogenesis and that multiple virulence factors may perform the same function. In infection development, *S. aureus* has numerous surface proteins, called "microbial sur-face components recognizing adhesive matrix molecules" (MSCRAMMs) that mediate adherence to host tissues (Patti *et al*., 1994). MSCRAMMs bind molecules such as collagen, fibronectin, and fibrinogen, and different MSCRAMMs may adhere to the same host-tissue component. MSCRAMMs appear to play a key role in initiation of endovascular infections, bone and joint infections, and prosthetic-device infections. Different *S. aureus* strains may have different constellations of MSCRAMMs and so may be subjected to causing certain kinds of infections (Patti *et al*., 1994; Foster and Hook, 1998).

Once *S. aureus* adheres to host tissues or prosthetic materials, it is capable to grow and persist in various ways. *S. aureus* can form biofilms (slime layers) on host and prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials (Donlan , *et al*., 2002). The ability to form and reside in biofilms is one reason why prosthetic device infections, for example, can be so difficult to eradicate without removal of the device. In vitro, *S. aureus* can also invade and survive inside epithelial cells, including endothelial cells, which theoretically may also allow it to escape host defenses, particularly in endocarditis (Arrecubieta and Lowy 2006; Moreillon *et al*, 2002).

S. aureus is also able to form small-colony variants (SCVs), which may contribute to persistent and recurrent infection. In vitro, SCVs are able to "hide" in host cells without causing significant host-cell damage and are relatively protected from antibiotics and host defenses. They can later revert to the more virulent wild-type phenotype, possibly resulting in recurrent infection (Proctor *et al*., 1995).

S. aureus has many other characteristics that help it evade the host immune system during an infection (Foster 2005) and its main defense is production of an anti-phagocytic microcapsule. The zwitterionic capsule (both positively and negatively charged) can also induce abscess formation (O'Riordan and Lee, 2004; Tzianabos *et al*., 2001). The MSCRAMM protein A binds the Fc portion of immunoglobulin (Deisenhofer, 1981) and, as a result, may put off opsonization. *S. aureus* may also secrete chemotaxis substance which is inhibitory protein of *staphylococci* or the extracellular adherence protein, which interfere with neutrophil extravasation and chemotaxis to the site of infection (Foster, 2005). In addition, *S. aureus* produces leukocidins that cause leukocyte destruction by the formation of pores in the cell membrane (Arbuthnott *et al*., 1990).

During infection, *S. aureus* produces many enzymes, such as proteases, lipases, and elastases, which enable it to invade and destroy host tissues and metastasize to other sites. *S. aureus* is also capable of producing septic shock. It does this by interacting with and activating the host immune system and coagulation pathways. Peptidoglycan, lipoteichoic acid, and a-toxin may all play a role (Lowy, 1998). In addition to causing septic shock, some *S. aureus* strains produce superantigens, resulting in various toxinoses, such as food poisoning and toxic shock syndrome (Dinges *et al*., 2000). Unlike the structural components noted earlier, these superantigens can produce a sepsis-like syndrome by initiating a "cytokine storm." Some strains also produce epidermolysins or exfoliative toxins capable of causing scalded skin syndrome (Prevost *et al*., 2003).

Regulation of expression of *staphylococcal* virulence factors plays a central role in pathogenesis. To reduce undue metabolic demands, expression occurs in a coordinated fashion only when required by the bacterium. Expression of MSCRAMMs generally occurs during logarithmic growth (replication), whereas secreted proteins, such as toxins, are produced during the stationary phase. During infection, the early expression of the MSCRAMM proteins facilitates initial colonization of tissue sites, whereas the later

elaboration of toxins facilitates spread. The accessory gene regulator (*agr*) is a quorum-sensing system that plays a critical role in the regulation of *staphylococcal* virulence (Yarwood and Schlievert, 2003). The *agr* mutants appear to have diminished virulence, and certain *agr* types are associated with particular clinical syndromes (Cheung *et al*., 1994).

Host factors may also affect susceptibility to *staphylococcal* disease but, in general, are poorly characterized. In general study indicated that those who are *S. aureus* carriers had higher mortality rate than non carriers. Because most infections with carriers occurred with their colonizing strains, colonization may confer some protective immunity if staphylococcal infection develops (Wertheim *et al*., 2004). Antibodies also appear to protect against the development of toxic shock syndrome, which occurs almost exclusively in those who lack antibodies to the implicated toxin at the time of acute illness (McCormick *et al*., 2001).

As described, *S. aureus* has numerous mechanisms to produce disease and to evade host defenses. However, it is important to note that not all *S. aureus* strains are developed equal. Different strains may contain different adhesins or toxins or may differ in their ability to produce biofilms and resist phagocytosis. The distribution of some virulence factors is related to clonal type, whereas the presence of others is unrelated to genetic background (Peacock *et al*., 2002). In this regard, it is important to note that there is limited information on the expression of these genes during infection.

1.2.5. The evolutionary history of Methicillin-resistant *Staphylococcus aureus*

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. For the first time in 1961 there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to Methicillin (MRSA), (Foster, 2005) and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States (Richard and Beigi, 2011).

MRSA is now a problem in humans and is increasingly recovered from nursing homes and the community. Though the origins of the major MRSA clones are still poorly understood; the Methicillin resistance gene (*mecA*) encodes a Methicillin-resistant penicillin-binding protein that is not present in susceptible strains and believed to have acquired from a distantly related species. *mecA* is carried on a mobile genetic element, called the *staphylococcal* cassette chromosome *mec* (SCC*mec*), of which four forms have been described that differ in

size and genetic composition (Hiramatsu *et al*., 2001). Many MRSA isolates are multiple resistant and are susceptible only to glycopeptides antibiotics such as Vancomycin and investigational drugs. MRSA isolates that have poor susceptibility to glycopeptides (glycopeptide intermediately susceptible *S. aureus*, (GISA)) (Hiramatsu *et al*., 2001), reported in recent years, are a cause of great public health concern.

1.2.6. Mechanism of Methicillin resistance

Intrinsic resistance to β-lactams in clinical strains of *S. aureus* is often heterogeneous. High-level resistance is expressed by a minority of cells on ordinary media at 37°C but more consistently in hypertonic media or at 30°C (Hartman and Tomasz, 1986). Although most MRSA produce a β-lactamase, this is not responsible for their resistance to methicillin. All MRSA contain the mecA gene and this is the essential determinant of methicillin resistance. *mecA* is a 2,130-bp segment of DNA coding for a penicillin binding protein (PBP2' or PBP2a) characterized by a low affinity for most β-lactams, and which is thought to take over the functions of all other PBPs when they are saturated by methicillin or other β- lactam antibiotics (Seligman,1966).

MSSA (Methicillin Sensitive *Staphylococcus aureus*) do not produce this protein and their DNA will not hybridize with a probe specific for the mecA gene. The genetic determinant of PBP 2a is transcribed in all MRSA cells and all phenotypic classes of MRSA, but additional factors affect the expression of meticillin-resistance (Seligman, 1966). The mecA gene is part of a mobile genetic element, the *staphylococcal* chromosomal cassette mec (SCCmec), which is incorporated in the chromosome. Five distinct types of SCCmec, designated I, II III, IV and V have been described to date. Most hospital-acquired MRSA are types I, II or III whereas most CA-MRSA are types IV or V (Kluytmans-Vandenbergh *et al*., 2006; Pantosti *et al*., 2007).

I. Enzymatic inactivation of the antibiotic

The most famous example of enzymatic inactivation of an antibiotic by bacteria, involves the neutralization of penicillin and penicillin-like antibiotics via the action of beta-lactamases. Beta-lactamases can be divided into four major groups based on primary structure alignments, molecular size, and active sites. Class-A enzymes harbor a serine in their active site and have an approximate molecular weight of 30Kda. Also, they are usually plasmid-

encoded and produce the Tumor Endothelial marker 1 (TEM-1) enzyme (Seligman, 1966; Peter, 1998).

Class-B enzymes are Zn^{2+}-metallo enzymes, and usually exhibit a broad spectrum of activity. Class-C beta-lactamases are chromosomally encoded, and, like their class-D counterparts, also harbour a serine in their active sites. Most beta-lactamases produced by gram-positive species are class-A enzymes. Approximately 90% of all beta-lactam resistant *S. aureus* produce beta-lactamases with the structural and regulatory genes being harboured by a plasmid (Peter, 1998).

II. Alteration of the antibiotic target

MRSA synthesize an additional Penicillin Binding Protein (PBP) which has a much lower affinity to beta-lactams than the normal PBPs and is therefore able to continue cell wall synthesis when the other PBPs are inhibited. Although, the mecA gene which codes for the additional PBPs is present on the chromosome in all cells of a resistant population, in many conditions it may only be transcribed in a proportion of the cells resulting in a phenomenon known as '' Heterogeneous resistance'' (Pantosti *et al*., 2007; John, 201). In the laboratory, special cultural conditions are used to enhance expression and demonstration resistance. MRSA are resistant to all other beta-lactams and the majority of strains produce beta-lactamase (John, 2011).

III. Restriction of antibiotic access to the target

It is common in gram-negative bacteria that beta lactams gain access to their target PBPs by diffusion through protein channels (porins) in the outer membranes. However, due to mutations in porin genes result in a decrease in permeability of the outer membrane and hence resistance. *S. aureus* Strains resistant by this mechanism may exhibit cross-resistance to unrelated antibiotics that use the same porins (Mins *et al*., 2004).

IV. Efflux Pumping

Efflux mechanisms have become broadly recognized as major components of resistance to many classes of antibiotics. Some efflux pumps selectively extrude specific antibiotics, while others, referred to as multidrug resistance (MDR) pumps, and expel a variety of structurally

diverse compounds with differing antibacterial modes of action (Lavigne *et al*., 2011). Antibiotic-specific efflux pumps are usually encoded on transmissible plasmids and transposons, while genes encoding many MDR pumps are normal constituents of bacterial chromosomes. Thus, *S aureus* bacteria have the potential to develop multi-drug resistance without acquisition of multiple specific resistance determinants (Lavigne *et al*., 2011; Van, *et al*., 2000).

Genes encoding some MDR pumps are expressed constitutively in wild-type cells. As a result these cells have basal levels of efflux activity, contributing to decreased antibiotic susceptibility. This intrinsic resistance may be low enough for the bacteria still to be susceptible to therapy. However, they would be even more susceptible if efflux pumps were rendered non-functional, allowing lower doses of antibiotics to be used in therapy. This could be especially important for antibiotics with narrow therapeutic indices (Hiramatsu, *et al*., 2001).

1.2.7. Epidemiology of MRSA in Pregnant Women

The spread of strains of MRSA acquired in the community and health institutions extensively reported in the USA and different parts of the world (Kazakova *et al*., 2005). However, the predominant CA-MRSA genotype in the US, known as USA300, strongly connected with skin and soft tissue infections; outbreaks are commonly associated with conditions of crowding, compromised skin, contaminated fomites, close contact, and lack of cleanliness. These factors are present in many settings and populations such as prisons, football teams, naval ships, military recruits, homeless persons, and homosexuality (Men to Men). Most purulent skin and soft tissue infections seen in emergency room settings are now caused by MRSA (Fridkin *et al*., 2005).

In several studies as shown hospital acquired methicillin *Staphylococcus aureus* (HA-MRSA) occurred in neonatal intensive care unit, but CA-MRSA is emerging in pregnant women and healthy term infants. Labor and delivery was the most second regular discharge diagnosis in the USA at just over 4 million births per year. In addition, ante-partum hospitalizations for pregnancy complications occur at a rate of 15 to 25 hospital admissions for every 100 deliveries (Bennett *et al*., 1998). A preliminary data for 2005 indicated that 12.7% of infants were born prematurely due to MRSA related cases. Implementing appropriate infection control measures in this large hospitalized population represents a major challenge, as

restriction of contact between mothers and infants is neither feasible nor desirable (Hamilton *et al*., 2007).

1.2.8. MRSA in Pregnant Women

It has been demonstrated that colonization with *S. aureus* predisposes to infectious morbidity, largely manifested as surgical-site infection. It has been supposed that the same risk exists (and potentially higher) among those with MRSA colonization. Exposure to children in daycare (Bennelt *et al*., 1998), and heterosexual transmission of CA-MRSA are additional potential sources of CA-MRSA acquisition and infection for women of childbearing age (Daum, 2007).

Numerous investigators have been studied the prevalence of MRSA colonization in pregnancy at varied anatomic sites and using different time-points. In 2006 studied nearly 3000 women during the third trimester and performed secondary cultures for *S. aureus* using the routine recto-vaginal cultures performed for identification of group B *streptococcus* (GBS) at 35–37 weeks of gestation in USA. Approximately 17% of women (507/2963) were positive for *S. aureus* colonization at this anatomic site. Only 14 (2.7%) of these 507 isolates were MRSA, leaving an overall rate of 0.5% MRSA rectovaginal colonization among these women. However, this higher colonization rate of *S. aureus* may be due to a result of additional detection of rectal *S. aureus* colonization. The increase in prevalence of *S. aureus* in this study noted may have also resulted from increased detection secondary to the use of a selective culture medium (Chen *et al*., 2006).

Investigators from Birmingham, Alabama, investigated the prevalence of genital tract MRSA colonization with pregnant women and its association with early-onset neonatal MRSA infection. Data on 5,732 pregnant women who were screened for *S. aureus* and group B *streptococcus* (GBS) between 35 and 37 weeks of gestation from July 2003 through June 2006 and their 5,804 infants (70 twin and 1 triplet sets) were reviewed. The maternal culture results were compared with a database of all neonates with a positive MRSA culture who were delivered to the mothers for the period of the study interval. Overall, maternal cultures were positive for GBS in 1,312 mothers (22.9%) and for *S. aureus* in 833 (14.5%). Of the 833 positive *S. aureus* cultures, 202 (24.3%) were MRSA, yielding a prevalence of MRSA in this population of 3.5% (Andrews *et al*., 2008).

Using a pilot study design, Beigi and Hanrahan cultured 104 samples from women entering labor and delivery for obstetric care in 2 different anatomic sites, and noted that 22% of women were colonized in the nares and 14.2% in the vagina with *S. aureus* which is almost similar with a study conducted in Birmingham, Alabama. Overall 9% of the isolates were MRSA, producing a rate of 2.1% MRSA vaginal colonization (Beigi and Hanrahan, 2007).

Another study was conducted in Colombia University medical Center from Feb. to July 2009. The study were assessed the trends in anovaginal MRSA colonization in pregnant women and denoted that in 2005, the prevalence of MRSA anovaginal colonization was 0.5% and in 2009, another study was conducted in the same study site with similar study population of 2921. Anovaginal samples examined for the trends in prevalence of MRSA among pregnant women. The prevalence of MRSA and over all prevalence *S. aureus* in the study time (2009) was 0.6% and 12.4% respectively; and the trends in prevalence of ano-vaginal colonization with MRSA from 2005 to 2009 in this study population almost remains stable (Karina *et al*., 2010).

Hundred pregnant women were included in a pilot study to determine the prevalence of nasal and vaginal colonization of *S. aureus* and the antibiotic susceptibility pattern of the isolates in pregnant women attending a maternity hospital in Cartagena, Colombia during the months of January - June 2009. From 100 pregnant women who enrolled in the study, 34 were colonized with *S. aureus*; 29 only in the nares, three only in the vagina, and two at both sites. A total of 36 *S. aureus* isolates were recovered, nine 9/36 (25%) of which were methicillin-resistant *Staphylococcus aureus* (MRSA), one was from the vagina; thus, the overall Vaginal colonization rate of *S. aureus* among pregnant women was 3% and 2% for both nasal and vaginal (Oscar *et al*., 2012).

In similar manner, a study was conducted from April to august 2006 in Nashville Tennessee (Eastern Central USA) to assess the frequency of recto-vaginal MRSA among pregnant women. The frequency of *S. aureus* and positive MRSA recto-vaginal culture among 250 pregnant women was 22% and 10.4% respectively. It was high when compared with other findings in different countries (Creech *et al*., 2007). This highest prevalence may be due to using couple of samples in a single study participants and socioeconomic difference among the community.

1.2.9. Post-partum infections due to MRSA

Infections presenting after delivery include mastitis progressing to breast abscess, furunculosis, cellulitis, and wound infection called post partum infections. In one case, an infected episiotomy site appeared to be the source of septic pelvic thrombophlebitis and septic pulmonary emboli, with wound, blood and sputum cultures all positive for MRSA with a community genotype (Totas *et al*., 2003).

Postpartum mastitis (PPM) occurs in as many as one third of breastfeeding women in the United States and leads to breast abscess formation in approximately 10% of cases (Barbosa *et al*., 2003; Foxman *et al*., 2002). Although breast milk cultures are not routine in PPM management, the growth of potentially pathogenic bacteria (such as β-hemolytic *streptococci* and/or *Staphylococcus aureus*) is associated with longer time to recovery and more frequent abscess formation (Osterman *et al*., 2000).

In September 2002, three women presented with cases of post-partum mastitis caused by MRSA that were reported to department of Epidemiology at New York–Presbyterian Medical Centre by obstetrical health care professionals. All 3 had delivered healthy, full-term infants in August 2002. Eight women were identified— including the initial 3 patients—who developed postpartum infections caused by MRSA (Saiman *et al*., 2003). All 8 case patients delivered their children during the same 2 weeks in August 2002, but the mean time to onset of MRSA infection was 23 days (median, 13.5 days; range, 4–73 days) after delivery. None of the newborn infants delivered by these 8 women were hospitalized in the neonatal intensive care unit (NICU), and none developed infections caused by MRSA during hospitalization or after discharge from the well-baby nursery (Saiman *et al*., 2003).

1.2.10. MRSA in newborns

Clusters of MRSA infections have been described in healthy newborns before or shortly after discharge from the hospital (Bartu *et al*., 2005). A more extended series of 61 cases of MRSA found a predominance of skin and soft tissue infections, but bacteremia, osteomyelitis, myositis, empyema, urinary tract infection, and one death were reported. Maternal skin infection has been noted in 21% of the records of infants with MRSA (Fortunov *et al*., 2006).

Study found that infants born to women colonized with *S. aureus* either during their third trimester of pregnancy or at the time of delivery are more likely to harbor *S. aureus* than are those born to non-colonized women. Infants born to mothers with *staphylococcal* colonization were nearly 4 times greater when mothers were also colonized than when mothers were not colonized (Peacock *et al*., 2003).

1.2.11. MRSA in Neonates Intensive Care Units (NICU)

Multiple outbreaks of MRSA have been reported from neonatal ICUs from the era prior to the emergence of CA-MRSA and more recently (Nguyen *et al*, 2007). Neonates are at risk of severe invasive disease from MRSA, including sepsis and death (Saiman *et al*., 2003). Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis (Bang *et al*., 1999) Sepsis is the commonest cause of neonatal mortality; it is responsible for about 30-50% of the total neonatal deaths in developing countries (Bang *et al*., 1999; Stoll, 1997).

It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes (Stoll, 1997). Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care. Neonatal sepsis can be classified into two major categories depending up on the onset of symptoms (Singh *et al*., 1994).

Early onset sepsis (EOS)*:* It presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract. Some maternal or perinatal conditions have been associated with an increased risk of EOS. Knowledge about these potential risk factors would help in early diagnosis of sepsis (Singh *et al*., 1994).

Late onset sepsis (LOS): It usually presents after 72 hours of age. The source of infection in LOS is either nosocomial (hospital-acquired) or community-acquired and neonates usually present with septicemia, pneumonia or meningitis (Baltimore, 1998). Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity,

admission in intensive care unit, mechanical ventilation, invasive procedures, administration of parenteral fluids, and use of stock solutions. Factors that might increase the risk of community-acquired LOS include poor hygiene, poor cord care, bottle-feeding, and prelacteal feeds. In contrast, breastfeeding helps in prevention of infections (Wolach, 1997).

The incidence of neonatal sepsis according to the data from Indian National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births (Stoll, 1997). The database comprising 18 tertiary care neonatal units- across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths (Stoll, 1997). Septicemia was the commonest clinical category with an incidence of 23 per 1000 live births while the incidence of meningitis was reported to be 3 per 1000 live births. Among births in this medical institution, *Klebsiella pneumoniae* was the most frequently isolated pathogen (32.5%), followed by *Staphylococcus aureus* (13.6%). Among neonates other than this institution (referred from community/other hospitals), *Klebsiella pneumoniae* was again the commonest organism (27%), followed by *Staphylococcus aureus* 15% and *Pseudomonas aeruginosa* 13% (Stoll, 1997).

1.2.12. Laboratory Diagnosis of MRSA

The definitive laboratory studies to diagnose that a person is infected or not with MRSA are straight forward. *S. aureus* is isolated and identified from the patient by standard microbiological techniques. Several selective media have been used for screening MRSA, such as Mannitol salt agar with oxacillin, Oxacillin blood agar and CHROM agar (Matos *et al*., 2010).

I. Phenotypic detection systems

S. aureus can be cultivated on Mannitol Salt Agar (MSA) or blood agar with B- hemolytic colonies at 35-37 ^{o}c for 24-48 hrs aerobically. The colonies will appear as golden yellow in MSA and white in blood agar. The isolated colonies are further identified by catalase test, coagulase test, and DNAse test (are positive for the three tests). Finally it will be subjected to further study using antimicrobial susceptibility test (Matos *et al*., 2010,).

Phenotypic expression of resistance can vary depending on the growth conditions (e.g. temperature, osmolarity and culture medium supplements such as NaCl or sucrose), making

susceptibility testing by standard microbiological methods potentially difficult. Out of the most phenotypic methods, agar dilution and disc diffusion are common (Matos *et al.*, 2010).

A. Agar dilution test

A minimum of four to five *S. aureus* colonies isolated from an overnight growth are transferred to sterile saline. The suspension is adjusted to a 0.5 McFarland standard (10^8cfu/ml) and spot inoculated on Mueller–Hinton agar plates supplemented with 2% NaCl and containing 0.125 μg oxacillin/ml in serial doubling dilutions. The oxacillin Mueller–Hinton plates are incubated at 35^Oc for 24 hours. MIC of ≥ 4 μg /ml is considered as resistant and MIC of ≤ 2 is considered as to be susceptible (Datta *et al*., 2011).

B. Disc diffusion test

A direct colony suspension of each *S. aureus* isolate is prepared to a 0.5 McFarland standard and plated on Mueller-Hinton agar containing 2-4% NaCl. An Oxacillin (1 μg) disk is placed on the surface and incubated at 35°C for 24 hours. Oxacillin disk is more resistant to degradation in storage and more likely to detect hetero resistant strains. The zone of inhibition must be read with transmitted light and not reflected light. Zone diameter of ≤10 mm is considered as resistant, ≥13 mm as susceptible whereas 11-12 mm is considered as intermediate, but the way of interpretation of the zone of inhibition may vary according to manufacturer guide line. If intermediate results are obtained for *S. aureus*, testing for *mec*A, PBP2a, cefoxitin disk test, oxacillin MIC test or Oxacillin-salt agar screen test may be performed. Any discernable growth within the zone of inhibition when seen using transmitted light is indicative of Oxacillin resistance. It may be possible that some of the Oxacillin disk test positive isolates are hyperbeta- lactamase producers, thereby accounting for non-*mec*A-mediated Methicillin resistance (Eksi *et al*., 2011).

In disc diffusion tests, hyper-producers of Penicillinase may show small Methicillin or oxacillin zones of inhibition, whereas most true Methicillin-/oxacillin-resistant isolates give of inhibition no zone. A 5μg Methicillin disk can also be used but is not a popular choice. Zone diameter of ≤9 mm is considered resistant, ≥14 mm is considered resistant whereas a diameter of 10-13 mm is considered intermediate (Eksi *et al*., 2011).

IV. Molecular methods

Detection of *mec*A gene by PCR is considered as the gold standard. DNA extraction is performed on the isolate and *mec*A gene is amplified using specific primers. The master mix containing PCR buffer, dNTP mix, primer, Taq DNA polymerase, and $MgCl_2$ and template DNA is subjected to hot start PCR. This is followed by 30 cycles of denaturation at 94^{o}C for 45 seconds, annealing at 50^{o}C for 45 seconds, and extension at 72^{o}C for 1 minute and final extension step at 72^{o}C for 3 minutes. PCR products are visualized on 2% agarose gel with ethidium bromide dye under UV trans-illuminator (Matos *et al*., 2010; Elizabeth and Mathew, 2011).

Another method that is designed to detect MRSA directly from clinical samples uses a frontend immune capture of *S. aureus* followed by MRSA detection using a multiplex PCR that detects *S. aureus* specific *fem*A and *mec*A. *S. aurous* specific targets used in various user-defined molecular assays for detection of MRSA (Dickson *et al*., 2011).

- *nuc* Encodes heat-stable DNA nuclease gene
- *fem*A, *fem*B Encode enzymes important in cross-linking peptidoglycan
- *spa* Encodes S. aureus-specific protein A

The hyplex StaphyloResist™ and hyplex StaphyloResist™ plus are qualitative multiplex PCR assays for the direct detection of clinically relevant *Staphylococci* from swabs of the nose, skin, wounds, vaginal, and tracheal specimens. The assay consists of PCR modules that contain labelled oligonucleotide primers, enabling simultaneous and specific amplification of different *Staphylococcal* DNA regions in a single PCR reaction. The PCR is followed by reverse hybridization procedures using single-stranded specific probes immobilized on micro-titre plates. Hybridization of PCR products with specific probes is detected using the ELISA principle (Gagliotti *et al*., 2012).

Detection of mecA gene or its product, penicillin binding proteins (PBP2a), is considered the gold standard for MRSA confirmation. Isolates of *S. aureus* that carry *mec*A gene or that produce PBP2a should be reported as MRSA and isolates lacking mecA gene or not producing PBP2a should be reported as Methicillin susceptible (Enright *et al*., 2002; Elizabeth and Mathew, 2011).

1.2.13. Treatment

Because MRSA is resistant to a number of different antibiotics, it is harder to treat than non-resistant bacteria. However, MRSA is not resistant to every antibiotic and most strains of MRSA can still be treated with vancomycin and others. However, there is no documented comparative investigation of therapy for MRSA infection in pregnancy. Treatment specifics in pregnancy are an important consideration for many reasons. Pregnancy often imparts many important physiologic changes to drug metabolism because of the known pregnancy-generated physiologic affects on the renal, gastrointestinal, hepatic, and hematologic organ - systems. These changes have the potential to alter therapeutic drug levels radically. An important concern must be taken during dealing with antimicrobial resistant pathogens such as MRSA. In addition, safety of available agents is always of concern when dealing with therapeutics in pregnancy. It should also be remembered that gestational-age specific safety concerns could influence antibiotic selection as agents are chosen to treat invasive infections in pregnancy from MRSA (NeVille and Clayton, 2010).

1.2.14. Prevention

A primary mode of transmission of MRSA is person-to-person via contaminated hands; therefore, the best way to avoid MRSA infection is not making direct contact with skin, clothing, or any items that are exposed to either MRSA patients or MRSA. This is often not possible because MRSA-infected individuals or MRSA carriers are not immediately identifiable. A first step is excellent hygiene practices (for example, hand washing with soap after personal contact or toilet use, washing clothes potentially in contact with MRSA patients or carriers, and using disposable items such as gloves when treating MRSA patients). MRSA may also be transmitted by sharing towels, personal hygiene items, and athletic equipment; through close-contact sports; and by sharing tattoo or injection drug use equipment, thus, antiseptic solutions and wipes are important in both clean hands and equipments that may contact MRSA. These are useful at home, in gyms, or almost any public place such as a public restroom (Broks 2007; CDC, 1999).

Another prevention method is to treat and cover (for example, antiseptic cream and a Band-Aid) any skin breaks. Pregnant women need to consult with their doctors if they are infected or are carriers of MRSA. Although MRSA is not transmitted to infants by breastfeeding unless the nipple(s) are infected, there are few reports that their MRSA-positive mothers can

infect infants, but this seems to be an infrequent situation. Some pregnant MRSA carriers have been successfully treated with the antibiotic (Richard and Beigi, 2011).

Caregivers to MRSA patients usually can avoid from infected by good hygiene (hand washing, using towels, linens and clothing that may contact the patient only once and then washing). When changing dressings or one is likely to contact body fluids, including saliva, urine and other body fluids; disposable gloves must be used. General screening of people is only recommended for high-risk patients who are being admitted to the hospital according to 2010 CDC guidelines. The infection-control group in hospitals usually does this. Some hospitals have already instituted this practice: since MRSA infections have begun to decrease, investigators suggest this practice, along with good home care (after diagnosis and treatment), is responsible for the decrease (CDC, 2004; Richard and Beigi, 2011).

1.3. Significance of the study

The epidemiology and clinical patterns of MRSA infections in the developed countries are well studied. MRSA has become an increasingly aggressive and prevalent pathogen in medicine. The clinical impact of MRSA colonization among pregnant women has estimated to be modest. In some studies, the impact of MRSA colonization is increasingly important pathogen in modern health care and in obstetric population. Roughly 357 invasive MRSA infections per 100 000 live birth in the United States occur annually (Richard and Beigi, 2011).

The African data on *S. aureus*, particularly on antibiotic susceptibility, are extremely limited, although Methicillin resistant *S. aureus* (MRSA) has disseminated in African countries (Taiwo *et al*., 2005). Between 1996 and 1997, the prevalence of MRSA, determined in some African countries, was relatively high (21 to 30%) in Nigeria, Kenya, and Cameroon (Kesah *et al*., 2003).

Studies from the United States, have reported that vaginal colonization rates for *S. aureus* in pregnant women ranged from 14% to 17.1%, and although the risk of vertical transmission has been suggested or even demonstrated (Beigi and Hanragan, 2007; Chen, et al., 2006).

In Ethiopia, different studies have been conducted in different parts of the country to identify and determine the drug susceptibility patterns of common pathogens. The results indicated that the presence of multi drug resistance from different clinical samples with the common isolates being *S. aureus* (Assefsa *et al*., 2008; Genet *et al*., 2012; Gebre-Selassie, 2007; Abera *et al*., 2008). This figure may not be described the burden of disease caused by MRSA in pregnant women.

Therefore, the present study was undertaken in order to investigate the epidemiology and the antimicrobial resistance pattern of *S. aureus* in pregnant women in Mekelle, North Ethiopia.

1.4. Objectives of the study

General objective

- To determine the genital colonization rate of *S. aureus*, Methicillin-resistant *S. aureus* and drug susceptibility pattern of among pregnant women attending antenatal care unit at Ayder Hospital, Mekelle.

Specific objectives

- To determine the prevalence of *Staphylococcus aureus* vaginal colonization in pregnant women.
- To determine the drug susceptibility pattern of *Staphylococcus aureus* vaginal isolates to commonly used drugs.

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CHAPTER-II: MATERIALS AND METHODS

2.1. Study Design and period

A hospital based prospective cross sectional study was conducted between December 1, 2011 to February 30, 2012 at Mekelle University Ayder teaching and referral hospital, Mekelle, Ethiopia.

2.2. Study Area

The study area was Ayder teaching and referral Hospital in Mekelle, Tigray Northern Ethiopia. It is located about 783 kilometers north of the capital, Addis Ababa, at latitude and longitude $13^0$29'N $39^0$28'E with an elevation of 2084 meters above sea level. The town has one teaching and referral hospital, one general hospital, and four health centers. According to the Ethiopia Central Statistic Agency the total population of the town in the year 2007 was about 298,000.

Ayder Hospital is a teaching and referral hospital of Mekelle University in the Health College sciences which started community service since 2006. It has about 500 beds and also it has more than 300 health workers and it gives service for an average of about 50,000 patients annually.

2.3. Study Participants and Sampling Method

The study participants were pregnant women at and beyond 24 weeks of gestation age attending the antenatal clinic unit of Ayder hospital. A total of 190 pregnant women were enrolled and investigated for *Staphylococcus aureus* vaginal colonization and drug susceptibility pattern of isoltaes.

The sample size is determined based on the prevalence rate of the study done by Beigi and Hanragan (2007). Non-probable convenient method was used and the sample size was determined by using the formula;

$$n = \frac{(Z\alpha/2)^2 (p\,[1- p])}{(d)^2}$$

$$\frac{(1.96)^2\ (0.142\ [1-0.142])}{(0.05)^2} = \mathbf{187} + \text{(10\% contingency)} = 206$$

Where, **n**: minimum sample size **d**: degree of accuracy desired (0.05)

P: prevalence of *S. aureus* (14.2%) (Beigi and Hanragan, 2007)

Histories were taken from each pregnant woman after written consent obtained from each of them before sample collection.

2.4. Sample Collection, Handling and Transportation

Vaginal swabs were collected by swabbing the vaginal vault (the outer third portion of the vagina) using a moisten, sterile swab from 190 pregnant women at and beyond 24 weeks of gestation by midwife nurses using single use swab from each after consent was obtained from the study participants . The principal investigator/s received the clinical samples (vaginal swabs) labeled with participant's number and time of collection in sample container. Swabs (vaginal) after taken were transferred to a sterile nutrient broth (Oxoid Ltd. UK) containing screw capped test tube. Separate sterile disposable gloves were used for each individual. Samples were transported to the laboratory for culture and identification of *S. aureus* immediately. Whenever there is delay in sample processing, Cary-Blair transport media was used (Oxoid Ltd. UK). Questionnaire was administered by the medical staff to each pregnant woman to collect demographic data and applicable medical, family and social history.

2.5. Culture and Identification

Vaginal swabs were inoculated directly on Mannitol Salt Agar (MSA) media (Oxoid Ltd. UK). Vaginal specimens on the sterile swab were rolled firmly over one-sixth of the labeled plate to deposit the specimen, then a sterile wire loop was used to streak the inoculum over the surface of the plate. Streaked culture plates were incubated at temperature of 37^{o}C aerobically for 18 to 24 hours (see Annex IV).

Then after 24 hours incubation, each plate was checked for growth and plates with growth were characterized by Mannitol fermentation (with small to large golden yellow pigment characteristics). Then after, colonies with Mannitol positive were selected and sub-cultured onto nutrient agar (Oxoid Ltd. UK) plates to obtain a pure growth. After overnight incubation

at a temperature of 37^{o}C, the pure colonies were tested for their gram reaction, catalase and coagulase test (Annex IV); finally the isolated *S. aureus* (based on Mannitol fermentation, catalase, and coagulase positive test) were subjected to antimicrobial susceptibility test to determine their drug susceptibility pattern (see Annex IV).

2.6. Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility pattern of the *S. aureus* isolates was determined using Kirby-Bauer-modified disc diffusion technique based on Cheesbrough (Cheesbrough, 2002) and Clinical and Laboratory Standards Institute (CLSI, 2009). Suspensions of *S. aureus* colonies were prepared by transferring pure 3-5 well isolated similar colonies of the organism to a tube containing physiological saline. The approximate turbidity of the suspension was adjusted visually to the optical density of 0.5 McFarland suspensions with equal amount (ml) to the test organism (see Annex IV). A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. Then, the bacterial suspension was spread gently over the surface of Mueller-Hinton agar plate (pH 7.2-7.4, Oxoid Ltd. UK).

The inoculated plates were left at room temperature to dry for 3-5 minutes. Then, antibiotic discs (Oxoid Ltd. UK) were applied on the surface of a Muller-Hinton agar plate using a disc dispenser (see Annex IV). Antimicrobial susceptibility testing was carried out on nine antimicrobials: Ampicillin (AMP) (10 µg), Amoxacillin (AMX) (2µg), Ciprofloxacin (CIP) (5 µg), Erythromycin (E) (15 µg), Gentamicin (GEN) (10 µg), Penicillin G (P) (10 U), Tetracycline (TE) (30 µg) Vancomycin (VA) (30µg), and Oxacillin (Ox) (1 µg) (Oxoid Ltd. UK).

Then plates were incubated at 37^{o}C for 18 to 24h under aerophilic atmosphere. The quality control for susceptibility was performed using the reference strain *S. aureus (ATCC 25923)*. After the overnight incubation, each plate was examined under an indirect light source from lamp to see the growth and inhibition zone around the disks. The zone of inhibition around the disc were measured diametrically using metal caliper and interpreted on the bases of the manufacturer instructions along the disk packages to classify as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI, 2009).

2.7. Reference Strains

S aureus (ATCC 25923) obtained from Ethiopia Health and Nutrition Research Institute was used as a positive control and all batches of freshly prepared media were tested for sterility by:

- Incubating representative un-inoculated plates and control organisms (ATCC 25923) inoculated on the media and incubated parallel to the specimens during culture and antimicrobial susceptibility of *S. aureus* tests.

- Applying the standard operational procedures (SOP) during each and every laboratory procedures and data collection from questionnaires.

2.8. Variables

2.8.1. Dependent Variables

- Methicillin resistance vaginal carriage
- Antimicrobial susceptibility pattern of *S. aureus* isolates

2.8.2. Independent variables

- Age , Gestation age , and Occupation

2.9. Data Entry, Management, and Analysis

Data obtained from the questionnaire and the laboratory result were entered to the computer, summarized, and analyzed using the Statistical Package Social Science program (SPSS), version 16.0 for windows. Frequencies of colonization were obtained and percentages were calculated for study variables. Chi-square and Fisher's exact test (two tailed) was used to calculate probabilities and $P < 0.05$ was used to determine statistical significance.

2.10. Ethical Considerations

The M.Sc. research project was ethically cleared by the Department Research and Ethical Review Committee (DREC) and approved by Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University. Official permission from the study site and written informed consent from study participants after they were informed about the study was also obtained (Annex III).

CHAPTER III: RESULTS

3.1. Demographic characteristics of study participants

During the study time period 190 pregnant women were screened for *S. aureus* and MRSA colonization using vaginal swabs culture. The study participants' age range was between 15 and 41 years with 25.2 years average age. One hundred and seventy five (92%) were from urban and 15 (8%) from rural areas. In terms of their marital status; One hundred and eighty (94.7%) of them were married; their occupational status revealed that 1.6% of the participants were health professionals, 6.8% were from other professions and one hundred and seventy four (91.5%) were house wife with different educational level (0 - 12 Grades). The gestational age range and gestational age mean of all study participants were between 24 to 42 weeks and 31.4 weeks respectively (Table 3.1).

Table 3.1: Demographic characteristics of the study population (n=190) investigated for *S. aureus* vaginal colonization at Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Variables		**Number (%)**
Age (Years)	14.5 – 25.5	107 (56.3)
	25.5 -.35.5	74 (40)
	35.5 – 45.5	9 (4.7)
(Age range 15 – 41 years)		
Address	Urban	175 (92)
	Rural	15 (8)
Marital status	Married	180 (94.7)
	Unmarried	10 (5.3)
Occupation	Health	3 (1.6)
	Other profession	13 (6.8)
	House Wife	174 (91.6)
History of Pregnancy	Primigravida	89 (46.8)
	Muligravida	101 (53.2)
Gestation age (in weeks)	23.5 – 27.5	61 (32)
	27.5 – 31.5	32 (17)
	31.5 – 35.5	44 (23.2)
	> 35.5	53 (27.8)
(Gestation age range: 24 – 42 weeks)		
Antenatal care visit	1 time	56 (29.5)
	2 times	74 (39)
	3 times	32 (17)
	4 times	17 (9)
	> 5 times	11 (5.5)

3.2. Rate of Vaginal colonization of *Staphylococcus aureus*

A total of 190 pregnant women were screened for vaginal colonization of *S. aureus.* Culture data were available from 184 (96.8%) of samples. Out of 184 pregnant women screened, 43 (23.4%) were colonized with *S. aureus* (Table 3.4). The age wise distribution of *S. aureus* in the study participants is shown in Table 3.2.

Of the total 43 *S. aureus* positive women, the majority 40(93%) of them were in the age range between 14.5 and 35.5 years and the remaining 3(7%) pregnant women were between 35.5 and 45.5 years.

Table 3.2: Frequency of vaginal colonization of *Staphylococcus aureus* in pregnant women at different age groups in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Age range (in years)	***Staphylococcus aureus***	
	Number	**(%)**
14.5 – 25.5	20	(46.6)
25.5 – 35.5	20	(46.6)
35.5 – 45.5	3	(7.0)
>45.5	-	
Total	**43**	**(100)**

Regarding to gestation age, all study participants' gestation age was between 24 and 42 weeks. Thirty six (84%) of the study participants with *S. aureus* were between 24.5 and 35.5 gestation weeks and the rest 7 (16.3%) were greater than 35.5 weeks of gestation (Table 3.3).

Table 3.3: Frequency of vaginal colonization of *Staphylococcus aureus* in pregnant women at different gestational ages in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Gestation age range (in weeks)	***Staphylococcus aureus***	
	Number	**(%)**
23.5 – 27.5	19	44.2
27.5 – 31.5	3	7.0
31.5 – 35.5	14	32.5
>35.5	7	16.3
Total	**43**	**100**

The distribution of *S. aureus* in terms of occupation was as follows, only 1/3 (33.3%) of mothers with *S. aureus* were health professional, 3/13 (23%) of mothers with *S. aureus* were other profession and 39/168 (23.2%) of the study participants with *S. aureus* were house wife pregnant women (Table-3.4). Of the total 43/184 (23.4%) *S. aureus* positive pregnant women, 11/43 (25.6%) had 1 time, 19/43 (44.2%) 2 times, 8/43 (18.6%) 3 times, and 5/43 (11.6%) 4 times visit to the ANC for follow up in the study site prior to this study.

Table 3.4: Frequency of vaginal colonization of *Staphylococcus aureus* in pregnant women in relation to their occupation in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Occupation	**No. of Sample collected**	***Staphylococcus aureus*** **No (%)**	***Staphylococcus aureus*** No (%) n=43
Health Profession	3	1 (33.3)	1 (2.3)
Other profession	13	3 (23)	3 (7.0)
House wife	168	39 (23.2)	39 (90.7)
Total	**184**	**43 (23.4)**	**43 (100)**

3.3. Antimicrobial susceptibility pattern of *Staphylococcus aureus*

The antimicrobial susceptibility pattern of *S. aureus* bacteria isolated from pregnant women against nine antimicrobial agents is presented in Table 3.5. In our study 43 *S. aureus* were subjected to nine antimicrobial agents. All the strains were resistant to ampicillin (100%), amoxicillin (100%), penicillin G (100%). High level of resistance was observed against tetracycline (83.7%). Low level (<60%) of resistance was observed for erythromycin (42%), followed by oxacillin (27.9%), ciprofloxacin (18.6%), gentamycin (4.6%), and vancomycine (2.3%).

Table-3.5: Resistance pattern of *Staphylococcus. aureus* isolated from pregnant women against 9 antimicrobial agents in Ayder Hospital, Mekele, Tigray (Dec. 2011 to Feb. 2012)

	Antimicrobial agents (% resistance)								
	AMP No. (%)	**AMX No. (%)**	**CIP No. (%)**	**E No. (%)**	**GEN No. (%)**	**OX No. (%)**	**P No. (%)**	**TE No. (%)**	**VA No. (%)**
S. aureus (n=43)	43 (100)	43(100)	8(18.6)	18(42)	2(4.6)	12(27.9)	43(100)	36(83.7)	1(2.3)

AMP: Ampicillin, **AMX**: Amoxycillin, **CIP**: Ciprofloxacin, **E**: Erythromycin, **GEN**: Gentamicin, **OX**: Oxacillin, **P**: Penicillin G, **TE**: Tetracycline, **VA**: Vancomycin

Multi-drug resistance was defined in this study as resistance to two or more of the antibiotics tested. Thus, all the 43 (100%) of the *S. aureus* isolates showed multi-drug resistance. Ampicillin, Amoxycillin, and Penicillin were the most frequently-occurring antibiotic in all the patterns of resistance combinations. One isolate was revealed full resistance to all tested antibiotics. And none of the 43 tested *S. aureus* was fully susceptible to all the tested antibiotics (Table 3.6).

Table 3.6: Prevalence of multiple-drug resistance among 43 *Staphylococcus aureus* isolates in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Types of drugs Resisted	**Frequency of Multi-drug Resistance n=43**	
	Number	(%)
AMP,AMX,P	4	9.3
AMP,AMX,P,E	1	2.3
AMP,AMX,P,TE	14	32.6
AMP,AMX,P,CIP,E	1	2.3
AMP,AMX,P,CIP,TE	1	2.3
AMP,AMX,P,CIP,OX	1	2.3
AMP,AMX,P,OX,TE	4	9.3
AMP,AMX,P,E,TE	8	18.6
AMP,AMX,P,CIP,E,TE	2	4.7
AMP,AMX,P,CIP,OX,TE	1	2.3
AMP,AMX,P,E,GEN,TE	1	2.3
AMP,AMX,P,E,OX,TE	3	7.0
AMP,AMX,P,CIP,E,OX,TE	1	2.3
AMP,AMX,P,CIP,E,GEN,OX,TE,VA	1	2.3
Total	**43**	**100**

AMP: Ampicillin, AMX: Amoxycillin, CIP: Ciprofloxacin, E: Erythromycin, GEN: Gentamicin, OX: Oxacillin P: Penicillin G, TE: Tetracycline, VA: Vancomycin

3.4. Rate of Methicillin resistance *Staphylococcus aureus* (MRSA)

When resistance was first described in 1961, methicillin was used to test and treat infections caused by *S. aureus.* However, Methicillin is no longer commercially available, and in many laboratories testing for methicillin resistance has been replaced by oxacillin, which is in the same class of drugs as methicillin. Therefore, in this study we have used oxacillin 1µg as a substitute for methicillin and the acronym MRSA is still used by many to describe these isolates because of its historic role.

Of the 184 pregnant women with culture data, 12 (6.5%) were colonized with MRSA. The drug resistance pattern of twelve MRSA isolates for eight antimicrobial agents is presented in Table 3.7. The drug susceptibility for the 12 MRSA isolates showed that a high level (>80%) of resistance to ampicillin (100%), amoxicillin (100%), penicillin G (100%), and tetracycline (91.6%). Low level (<60%) of resistance were observed for erythromycin (41.6%), followed by ciprofloxacin (33.3%), and 8.3% for gentamicin and vancomycin.

Table 3.7: Drug susceptibility pattern of MRSA isolates from pregnant women against 8 antimicrobial agents in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

	Antimicrobial agents (% resistance)							
	AMP No. (%)	**AMX No. (%)**	**CIP No. (%)**	**E No. (%)**	**GEN No. (%)**	**P No. (%)**	**TE No. (%)**	**VA No. (%)**
MRSA (n=12)	12 (100)	12(100)	4(33.3)	5(11.6)	1(8.3)	12(100)	11(91.6)	1(8.3)

AMP: Ampicillin**, AMX:** Amoxycillin**, CIP:** Ciprofloxacin**, E:** Erythromycine**, GEN:** Gentamicin**, , P:** Penicillin G**, TE:** Tetracycline**, VA:** Vancomycin

3.5. Antimicrobial Susceptibility patterns of MRSA and MSSA isolates

The antimicrobial susceptibility patterns of MRSA and MSSA isolates to different classes of antibiotics are summarized in Table 3.8. All the 12 MRSA isolates were resistant (100%) to ampicillin, amoxycillin, and penicillin G, followed by tetracycline (91%), and less resistance rate was observed against erythromycin (41.6%), ciprofloxacin (33.3%), gentamicin (8.3%), and vancomycin (8.3%). Whereas, MSSA isolates showed moderate resistance to tetracycline (61.2%) followed by less similar to erythromycin (38.7%), Ciprofloxacin (12.9%), and gentamicin (3.2%). However, all MRSA and MSSA isolates tested in this study were recorded as resistant (100%) to ampicillin, amoxicillin, and penicillin. All MSSA isolates were found to be sensitive to vancomycin (100%) table 3.8.

Table 3.8: Antibiotic resistance pattern of MRSA and MSSA isolated from pregnant women in Ayder Hospital, Mekele, Tigray (December 2011 to February 2012)

Antibiotics	Percent of isolates resistance to antibiotics			
	MRSA n=12	MRSA (%)	MSSA n=31	MSSA (%)
Ampicillin	12	100	31	100
Amoxacillin	12	100	31	100
Ciprofloxacin	4	33.3	4	12.9
Erythromycin	6	41.6	12	38.7
Gentamicin	1	8.3	1	3.2
Penicillin G	12	100	31	100
Tetracycline	11	91	19	61.2
Vancomycin	1	8.3	0	00

CHAPTER-IV: DISCUSSION

Humans are a natural reservoir of *S. aureus*, and persons colonized with *S. aureus* are at an increased risk of becoming infected with these strains. In pregnant women, *S. aureus* causes a health threat because; it is the major cause of infection of the surgical site, causing of infections of the post cesarean surgical site, representing a major cause of morbidity and a cause of puerperal mastitis (Laibl *et al*., 2005). Early epidemiological studies showed that 5% of women were colonized with *S. aureus* in their genital tract and postpartum women had the highest colonization rates. Although risk factors associated to colonization with MRSA strains during pregnancy have not been fully characterized; associations with race, parity, type of birth, and colonization with group B streptococci have been suggested (Chen *et al*., 2007). Carriers of *S. aureus*, mainly methicillin resistant *Staphylococcus aureus* (MRSA), have a higher risk for increasing clinical infections, being the infections caused by MRSA strains the most important at the clinical level because they are more difficult to treat (Safdar, and Bradley, 2008). The role of MRSA carriers in the transmission of these pathogens to their babies is serious. In an early study reports have suggested that a potential for transmission of MRSA from mothers to infants. Nearly 10% of mothers and 2.5% of infants were colonized with MRSA at the time of delivery and colonization were nearly 4 times greater when mothers were also colonized than when mothers were not colonized (Chen *et al*., 2007).

The central focus of this study was on pregnant women because of the inevitable vital role they play in human race as wives and mothers. This is the first study, to our knowledge, to determine the rate of vaginal colonization *S. aureus* and MRSA in pregnant women in our country. We have described the prevalence of 23.4% *S. aureus* vaginal colonization in pregnant women. This was in line with reports in USA that found 17.1 and 22% of colonization (Chen, *et al*., 2006; Beigi and Hanragan, 2007 respectively. Similarly it was in agreement with the previous reports from Addis Ababa, Ethiopia which showed 20% *S. aureus* colonization among pregnant women (Assefa *et al*., 2008). This was higher than reports in USA (Karina *et al*., 2010) and France (Nadege *et al*., 2010) which revealed 12.4% and 5.9% respectively.

The increase in prevalence of *S. aureus* colonization that we noted may have also resulted from the use of a selective and differential culture media. Certainly, information from previous studies on the microbial flora of the lower female genital tract has been noted that

isolating microorganisms could be weakened by technical limitations, like failure to use the proper media, taking insufficient sample, missing the actual body sites during sample collection (Larsen and Monif, 2001). In addition, the risk factors for high colonization rate of *S. aureus* in pregnancy were not largely characterized. However, other studies elsewhere in the world indicated that difference in socio demographic factors might be the reason for genital colonization variation (Linnemann *et al*., 1982).

The antimicrobial susceptibility pattern of *S. aureus* isolates varies with place and time. In majority of the studies conducted over the years, there was a clear indication of the progressive development of antimicrobial resistance to several antibiotics (Qureshi *et al*., 2004). In our study antimicrobial susceptibility among *S. aureus* isolates was done against nine selected antimicrobials. The result showed that 100% resistant to ampicillin amoxycillin and penicillin and it is comparable with the study in Nigeria (Onanuga *et al*., 2005). High resistance were also observed to tetracycline (83.7%), followed by less resistance erythromycin (42%) oxacillin (27.9%), ciprofloxcillin (18.6%), gentamicin (4.6%), and vancomycin (2.6%).

The rate of resistance reported for tetracycline (83.7%) in this study was similar with reported (71%) in Ethiopia (Abera *et al.,* 2010). It is also comparable to those reported from other developing countries, the resistance rate to tetracycline varied from 88% to 100% (Durgadas *et al*., 2009; Adebayo *et al*, 2011).

Many factors might have contributed to such level of resistance, including misuse of antibiotics by health professionals, unskilled practitioners and laypersons. In Ethiopia, it is a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public and this contributing to the emergence and spread of antimicrobial resistance (Gemechu *et al*., 2011). Other causal factors might be poor drug quality, poor hospital hygienic conditions accounting the spread of resistant bacteria, and inadequate surveillance, i.e. lack of information from routine antimicrobial susceptibility testing of bacterial isolates and surveillance testing of bacterial isolates and surveillance of antibiotic resistance, all of which are crucial for good clinical practice and for rational policies against antibiotic resistance.

The present study indicated that the resistance of *S. aureus* to erythromycin was 42%. This result was higher than the resistance rate (23%) in Eritrea (Durgadas *et al*., 2009). In our

study the resistance rate of ciprofloxacin among pregnant women was 33.3%. This result was comparable with the study noted (29.4%) in Nigeria (Adebayo *et al*, 2011). However, it is much higher than the previous study reported (5%) in Eritrea (Durgadas et *al*., 2009).

In some of the studies, high resistance was reported in *S. aureus* isolates to gentamicin. And ranged from 14.7% to 32%, (Adebayo *et al*, 2011; Durgadas *et al*., 2009), whereas in our study the resistance rate to gentamicin was only 8.3%; and it was less than these previous studies.

According to the present study, the resistance rate of *S. aureus* to vancomycin was 2.6%. It is comparable with the study result reported in Colombia with resistance rate 0% (Karina et al., 2010). In contrast, our result was much lower than reported (61.1%) in Nigeria (Onanuga *et al*., 2005). Several conditions like social, economical, and cultural aspects might be the reason for variation in drug susceptibility to vancomycin.

The overall frequency of MRSA in our study was 12/184 (6.5%) and it was lower than reported in Nashville (Eastern central USA) 10.4% (Creech *et al*., 2007). However, our finding was higher than reported previously in Alabama, 3.5% (Andrews *et al*., 2008) USA, 2.1% (Beigi and Hanragan, 2007) and in columbia 1 % (Oscar *et al*., 2012). Geographic location, socioeconomic status and/or ethnic composition of the study participants and sample source (only vaginal swabs in our study) might be one factor for variation in colonization.

The prevalence of MRSA from the 43 *S .aureus* isolates in our study was found to be 27.9%. Our result was comparable with the studies made in Colombia 33.3% (Oscar *et al*., 2012) and Albama 24.3% (Andrews *et al*., 2008). However, it was markedly also than the previous studies conducted in USA 7% (Beigi and Hanragan, 2007), France 6% (Nadège *et al*., 2010), Columbia 4.9% (Karina et al., 2010), and in USA 2.7% (Chen *et al*., 2006). It was also much lower than the findings of Genet *et al.,* who reported 100% resistance in floor and tabletop surfaces of operation rooms and surgical wards in Jimma, Ethiopia (Genet *et al*., 20120) and the study reported 53.7% in Nashville (Creech *et al*., 2007). The difference might be due to implementation of selective media and use or not use of vaginal swabs with other body sites for the isolation of MRSA in addition to socio economic and geographical variation and the varied health status of subjects in the studies.

The drug susceptibility pattern of MRSA isolates also varies with time and geographic location. In our study antimicrobial susceptibility among MRSA isolates was done for 8 selected antimicrobial agents. The result showed that 100% resistance to ampicillin, amoxicillin, and penicillin. High resistance also were observed in tetracycline (91.7%) followed by erythromycin (41.6%), ciprofloxacillin (33.3%), gentamicin (8.3%) and vancomycin (8.3%). It was also comparable with most studies findings (Durgadas *et al*., 2009; Onanuga *et al*, 2005).

According to several studies reported, there was a clear development of antimicrobial resistance to different drugs through time. In our study over all multidrug resistance among MRSA isolates was higher than those that were MSSA (43.7% vs. 23.3%). This might be the presence of chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein (PBP2a) which has a low affinity for binding β-lactam antibiotics in MRSA strains (Weems, 2001).

The antimicrobial susceptibility for tetracycline was found to be 91.7 % among MRSA isolates. It was comparable with the result noted (78%) in Eritrea (Durgadas *et al*., 2009), however, it was much higher than reported (16.3%) in Colombia (Karina et al., 2010) and (55.9%) Nigeria (Adebayo *et al*, 2011).

The resistance rate for erythromycin in this study was 41.6% and it was lower than the study reported in colombia (83.3%) (Karina et al., 2010) and in Nigeria (54.5%) (Adebayo *et al*, 2011) but it was higher than noted (27%) in Eritrea (Durgadas *et al*., 2009). Resistance rate (33.3%) to ciprofloxacin in this study was observed in MRSA isolates from vaginal swabs. The rate was much higher than the results (8%) reported from Eritrea (Durgadas *et al*., 2009); however, it was largely lower than noted (72.7%) in Nigeria (Adebayo *et al*, 2011). The possible reason for the wide variation in resistance might be the improper utilization of the drug for the treatment of MRSA related causes and the varied health status of subjects in the studies.

The resistance pattern of individual MRSA isolates in a number of studies was (46 to 81%) resistance rate for gentamycine (Durgadas *et al*., 2009; Adebayo *et al*, 2011). In contrast, in our study the resistance rate for gentamycine was 8.3% and it was much lower than the study reported 81.5% and 46% in Nigeria (Adebayo *et al*, 2011) and Eritrea (Durgadas *et al*., 2009)

respectively. In our study, resistance rate for vancomycin was found to be 8.3% which was very less compared to previous reports done in Nigeria (89%) (Onanuga *et al*, 2005).

Conclusion and Recommendation

Conclusion

This study has provided data on the vaginal carriage rate of Methicillin resistance *Staphylococcus aureus*, and initial information on the prevalence of antibacterial resistance in *S. aureus* obtained from selected pregnant women. According to this study, high level of MRSA and multi drug resistance was observed among MRSA isolates from pregnant women. However, some of the tested *S. aureus* isolates show less resistance (8.3%) to vancomycin and ciprofloxacin; therefore these antimicrobial agents can be considered as the only agents to be less resisted by the isolates even with multi drug resistance.

Recommendation

Based on the findings in this study the following recommendations are made:

- The prevalence of MRSA in this study was high. Therefore, periodic studies are recommended to strengthen the findings of this study and also to observe any changes in the susceptibility patterns of *S. aureus* vaginal colonization in the pregnant women.
- In this study we found significant number of MRSA and the drug susceptibility pattern might not predict the source of these strains. Therefore, molecular characterization of MRSA is very important to trace source whether it is from hospital associated or community associated.
- It is recommended that treatment of MRSA infections should be based on culture and sensitivity. Therefore, the diagnostic capabilities of the Microbiology laboratory and laboratory personnel skills should be supported and strengthened.
- Infection control measures such as infection control programs in this area should be implemented, to hold more effectively the high prevalence of MRSA infections.
- Vancomycin and ciprofloxacin use should be limited to those cases where there are clearly needed.
- Hence, there is a need to adopt strategies to encourage proper personal hygiene

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ANNEX I

Questionnaire for investigation of the Colonization rate of MRSA among pregnant women in Ayder Teaching and Referral Hospital Mekelle, Ethiopia

a. Participant Identification

1. Serial No……. 2. Card no…….
3. Address …….. 4. Participant name……
5. Age…….. 6. Marital status……
7. Occupation……..
8. History of primigravida or multigravida: Primigravida ...……..
   Multigravida... … ……
   Gestational age (weeks)……
9. Number of prenatal visit……...
10. History of recent any antibiotic treatment Yes/No
    If yes, mention antibiotic/s and
    Time taken…….
11. History of any contraceptive use Yes/No
    If yes, mention type of contraceptive used…….
12. Date and time of vaginal specimen collection ……

b. Laboratory data

1. Growth of *S. aureus* on Manitol salt Agar selective media……

2. Result of Gram stain……

3. Catalase test result: positive/Negative

4. Coagulase test result: positive/Negative

5. Antimicrobial susceptibility test	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Ampicillin (10 µg)	____	____	_____
Amoxicillin (2 µg)	____	____	_____
Ciprofloxacin (5 µg)	____	____	_____
Erythromycin (15 µg)	____	____	_____
Gentamycin (10 µg)	____	____	_____
Oxacillin (1 µg)	____	____	_____
Tetracycline (30 µg)	____	____	_____
Penicillin G (10 U)	____	____	_____
Vancomycin (30 µg)	____	____	_____

Comments_____

_____

Name of principal investigator _____

Signature _____ Date _____

ANNEX II

INFORMATION SHEET FOR STUDY SUBJECTS

You are kindly invited to participate in this study, which involves about 206 pregnant women from Ayder Referral Hospital. The aim of this study is to determine prevalence of Genital colonization with Methicillin Resistant *Staphylococcus aureus* in pregnant women. Pregnant women with Methicillin Resistant *Staphylococcus aureus* colonization during 24 and beyond weeks of gestations will pose a risk to their children during delivery. Infection with this organism can cause different disease in new born and Mother; therefore, this study will identify colonization rate in pregnant women so that those who are colonized with Methicillin Resistant *Staphylococcus aureus* will receive prophylaxis or medication before delivery to keep their baby safe.

a. Purpose: the purpose of this research study is to assess the prevalence of colonization of Methicillin Resistant *Staphylococcus aureus* among pregnant women attending antenatal clinic in Ayder Referral Hospital, Mekelle, Ethiopia.

b. Duration: the duration of this study probably takes about three or four months or more.

c. Procedures to be carried on: the procedure of sample collection is easy and straight forward; sample will be collected from vagina area using cotton swab by attending physician or mid wife nurses and then it will be analyzed in the microbiology laboratory of Ayder Referral Hospital for the presence and isolation of Methicillin Resistant *Staphylococcus aureus*

d. Risk and discomfort: almost there will not be any risk associated during sample collection without little discomfort.

e. Expected benefits: from this study you will be benefited, as screening and from reduce the risk of vertical transmission of Methicillin Resistant *Staphylococcus aureus* to your infants.

f. Confidentiality: All your personal information collected for the purpose of the present study will be kept confidential.

g. Compensation: No compensation will be provided by participating in this study.

h. Termination of the study: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to

- Keep hold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study will be approved by Department Ethical and Review Committee and ethically cleared by Department of Microbiology, Immunology, and Paracytology, Faculty of Medicine Addis Ababa University.

If you have any question about the right of the study participant the address is:
Faculty of Medicine Addis Ababa University
Office of Associate Dean, Postgraduate Programs and Research
P.O. Box 9086. Addis Ababa, Ethiopia
Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:
Getachew Melkamu
Department of Microbiology, Immunology and Parasitology
Faculty of Medicine, Addis Ababa University
P.O.Box. 9086, Addis Ababa, Ethiopia
Tel: 0911859485

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ

ሀ . የጥ ናቱ ዓላማ፡- የዚህ ጥናት ዓላማ የስታፊሎ ኮከስ ኦሪስ ሜቴሲሊን መድሃኒት ለመድ ባክቴሪያን በነፍሰጡር እናቶች ላይ ያለውን ስርጭት ማጥናት ነው፡፡

ለ. የሚፈጀው ጊዜ፡- ይህ ጥናት ሦስት ወይም አራት ወር ሊፈጅ ይችላል፡፡

ሐ. አጠቃቀም፡- በዚህ ጥናት ከሚ ሳተፉ እናቶች ከሀፍረተ ሥጋ ላይ በህክምና ወይም በሚድዋይፈሪ ነርሶች ናሙ ና በመውሰድ ከላይ የተጠቀሰውን ባክቴሪያ መኖሩ ይረጋገጣል፡፡

መ. ሊደርስ የሚ ችል አደጋ፡-በዚህ ጥናት ውስጥ በጣም ትንስ ከሆነ አለመ መቸት በስተቀር አደጋ የሚያደርስ ድርጊት የለም።

ሠ. የሚገኝበት ጥቅም፡-ከዚህ ጥናት በተለይ የሚወለዱ ህፃናት ከፍተኛ ተጠቃሚ ይሆናሉ፡፡ ይህም ሊሆን የሚ ችለው ፤ ከላይ የተጠ ቀሰው ባክቴሪያ የተገኘባቸው እናቶች ቅድመ ህክምና በመ ው ሰድ ወደ ልጆቻቸው እንዳይተላለፍ በማድረግ ነው፡፡ እንዲሁም ለዚህ ባክቴሪያ ለተጠቁ እናቶችም ሆነ ህፃናት የተሸለ መድኃኒት እንዲያገኙ ይደረጋል፡፡

ረ. ሚስጥራዊነት፡-የማንኛውም የጥናቱ ተሳታፊ መረጃ በሚስጥራዊነት ይይዛል፡፡ የእያንዳንዱን ግለሰብ መረጃ ከዋናው ተመራማሪና አማካሪው በስተቀር ማንም ሊያገኝ አይችልም፡፡

ሰ. ፈቃደኝነትን ስለማቋረጥ፡-የጥናቱ ተሳታፊዎች፤መረጃ ያለመስጠት፣በጥናቱ ለመሳተፍ ፈቃደኝነት ያለማሳየት እንዲሁም ናሙና ያለመስጠት መብታቸው የተጠበቀ ነው፡

ይህ የጥናት ምርምር ከ አ.አበባ ዩኒቨርስቲ የህክምና ማይክሮባዬሎጅ ፡ ኢሚዩኖሎጅና ፓራሳይቶሎጅ የጥናት መመዘኛና ያማላ በመሆኑ ፍቃድ ማግኘቱን ላረጋግጥልዎት እወዳለሁ፡፡

አድራሻ ማወቅ ካስፈለግዎ፡-

ህክምና ፋክሊቲ፤አዲስ አበባ ዩኒቨርሲቲ

የድህር ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ.251-011-551-28-765

የዋናው ተመ ራማ ሪ አድራሻ፤

ጌታቸው መልካሙ

ማ ይክሮ ባዮሎ ጂ፤እም ኖሎጂ እና ፓረሳይቶሎ ጂ ትምህርት ክፍል

ህክምና ፋክሊቲ፤አዲስ አበባ ዩኒቨርሲቲ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 0911859485

ANNEX III

CONSENT FORM

Name…….…….. Card no…..……Serial no…….

I have been informed that the objective of this study is to determine the prevalence of *S. aureus* bacterial agent and pattern of antimicrobial resistance. The aim of the study is explained to me. The results of this study have importance to treat pregnant mothers and neonates who have MRSA and according the result profile, which guide the Gynecologists and pediatrician to manage the cause. I have also informed about the confidentiality of the questionnaires. Because, I have requested to participate in the study, which would require my response to an interview, and to provide vaginal swab, if there is suspected Methicillin Resistant *Staphylococcus aureus*. Therefore, with full understanding of the importance of the study, I agreed voluntarily to give the requested samples in the above for clinical investigation in the study and I benefit only from the free laboratory investigation result.

I_____ hereby give my consent for giving of the requested information and vaginal swab specimen as the doctors find best for me.

Signature: _____ Date_____

Principal investigator

Name_____Date_____

<u>የፈቃደኝነት መጠየቂያ ቅጽ</u>

ተራ ቁጥር.....የካርድ ቁጥር.....

የዚህ ጥናት ዋና ዓላማ የስታፊሎኮከስ አሪስ ሜቴሲሊን መድሃኒት ለመድ ባክቴሪያን (Methicillin Resistant *Staphylococcus aureus)* በነፍሰጡር እናቶች ላይ ያለውን ስርጭት ማጥናት ነው።በ24 እና ከዚያ በላይ የእርግዝና ሳምንታቸው ላይ የሚገኙ ነፍጡር ሴቶች ከዚህ በላይ የተጠቀሰውን ባክቴሪያ በሀፍረተ ሰጋቸው ከተሸከሙ፤ በእናቲቱ ላይና በሚወለደው ልጅ ላይ ችግር ሊፈጥር ይችላል። በዚህ ባክቴሪያ የተጠቁ እናቶችና ህፃናት የተለያዩ የበሽታ ምልክቶች ሊያሳዩ ይችላሉ። ስለዚህ በዚህ ጥናት የስታፊሎኮከስ አሪስ ሜቴሲሊን መድሃኒት ለመድ ባክቴሪያን (Methicillin Resistant *Staphylococcus aureus*) በሀፍረተ ሰጋቸው የተሸከሙትን እናቶች ማወቅና የባክቴሪያውን ስርጭት በማወቅ ቅድመ ጥንቃቄ ወይም ህክምና እንዲወስዱ ለማድረግ ነው። በዚህ ጥናት ውስጥ ምንም አይነት ጎጂ ድርጊት የለም፣እንዲሁም ማንኛውም መረጃ በሚስጥር ይያዛል። ስለዚህ በዚህ ጥናት በመሳተፍ አስፈላጊውን ናሙና /ከሀፍረተ ሥጋ/ እንዲወሰድና እንዲሁም ሊቃለ መጠየቅ እንድትተባበሩኝ እጠይቃለሁ።

እኔ_____ ከላይ የተጠቀሰውን አድምጩ ለእኔ ጠቃሚ መሆኑን ስለተረዳሁ በጥናቱ በመሳተፍ የሚፈለግብኝን ለማድረግ ተስማምቼለሁ።

የጥናቱ ተሳታፊ ፊርማ _____ ቀን_____

የተመራማሪው ፊርማ _____ ቀን_____

ANNEX-IV

Laboratory Procedures

1. **Culturing**

NOTE: The Media (Manitol Salt Agar and Nutrient Agar) were Prepared as per the manufacturers guide line.

Inoculation of Plates and Incubation

1. Prior to inoculation, the media should be brought to room temperature
2. Vaginal swabs rolled firmly over one-sixth of the plate to deposit the specimen.
3. A sterile loop used to carefully streak the inoculums over the surface of the plate and the plate incubated at 35^{o}C.
4. Finally, after 18h - 24h the plate will be examined for colony characterization and identification.

2. **Gram stain**
   1. Prepare a smear from the culture and heat gently to fix**.**
   2. Flood the slide with 0.5% methyl/crystal violet and leave for 1 min.
   3. Tilt the slide; pour on sufficient (1%) Lugol's iodine to wash away the stain, cover with fresh iodine and allow acting for 1 min.
   4. Tilt the slide and wash off the iodine with 95 - 100% ethanol or acetone until color ceases to run out of the smear or for 30 sec.
   5. Rinse with water.
   6. Pour on 0.1% counter stain (neutral red, safranin) and leave to act for about 2 min.
   7. Wash with water and blot dry.
   8. Examine under a microscope with 100x magnification power.

3. Catalase Test

- Using a Pasteur pipette 2-3 drops of hydrogen peroxide (3%) solution will be placed on a microscopic slide.
- Using a loop, suspected colonies will be picked without touching the plate and immersed in the hydrogen peroxide solution.
- Then the suspension checked for immediate bubbling. Active bubbling indicates a Positive test.

4. Coagulase test

Slide Test Method

- Add 10 µl of deionized water to slide or black card.
- Emulsify several colonies in to the water to obtain a smooth milk colored suspension.
- Add 1 to 3 µl of rabbit plasma and observe for clumping immediately, not to exceed 10s.

Interpretation

- A positive test is the demonstration of agglutination of the bacteria cells after the plasma is added.
- A negative test demonstrated by the lack of agglutination.

Tube test Method

- Bring tube of Rabbit Plasma to 25^{o}c.
- Inoculate with one colony of *Staphylococcus aureus* growing on non inhibitory medium or 2 drops of blood from a positive culture.
- Incubate at 35^{o}c without CO_2 for up to 4 h and observe for clot formation. Do not agitate the tube during observation.
- Incubate for an additional 20h at 25^{o}c for late clot formation.

NOTE: don't let the tube at 35^{o}c for more than 4 h since *S. aureus* fibrinolysin can lyse the clot.

Interpretation

A positive test meets one of the following criteria

- Complete clot formation or any degree of clot formation before 25 h

- No clot formation after addition of 1 or 2 drops of 5 % $CaCL_2$ to a tube without a clot at 24 h.

A negative test meets the following criteria

- A lack of clot formation at 24 h at 25^{o}c.
- No clot after 24 h at 35^{o}c, but after addition of 1 or 2 drops of 5% Ca CL_2 to the tube, a clot forms.

5. Antimicrobial susceptibility test

Kirby-Bauer CLSI (NCCLS) modified disc diffusion Technique.

Preparation of turbidity standard

Turbidity standard equivalent to McFarland 0.5

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid ***to*** 99ml of water. Mix well.

Caution**:** Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and *never add the water to the acid*.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water.
3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.
4. Transfer a small volume of the turbid solution to a capped tube or screw cap bottle of the same type as used for preparing the test and control inocula.

When stored in a well-sealed container in the dark a room temperature (20–28^{o}c), the standard can be kept for up to 6 months.

Procedure

1. Using a sterile wire loop, take 3–5 well-isolated pure colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.

3. Using a sterile swab inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o} to ensure even distribution.
4. With the petridish lid in place, allow 3–5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.
5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate.

   ***Note*:** The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35^{o}c for 16–18 h (temperatures over 30^{o}c invalidate results for Oxacillin).
7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my own original work, has not been presented for a degree or another purpose in any other University or institutions and that all sources of materials used for the thesis have been duly acknowledged.

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