INTRODUCTION

*Staphylococcus aureus* is a bacterium that is frequently found in the human respiratory tract and on the skin. Although *Staphylococcus aureus* is not always pathogenic, it is a common cause of skin infections, respiratory disease and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of methicillin resistant forms of pathogenic *Staphylococcus aureus* (MRSA) is a worldwide problem in clinical medicine (Kwan, 2005).

*Staphylococcus* was first identified in Aberdeen, Scotland (1880) by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint (Ogston, 1984). This name was later appended to *Staphylococcus aureus* by Rosenbach who was credited by the official system of nomenclature at that time. It is estimated that 20% of the human population are long-term carriers of *Staphylococcus aureus* (Kluytmans, 1997) which can be found as part of the normal skin flora and in anterior nares of the nasal passages (Kluytmans, 1997; Cole *et al.*, 2001). *Staphylococcus aureus* is the most common species of staphylococcus to cause *Staph* infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies (Kluytmans, 1997; Cole *et
al., 2001). *Staphylococcus aureus* can cause a range of illnesses from minor skin infections such as pimples, impetigo, boils, furuncles, cellulitis, folliculitis, carbuncles, Scalded Skin Syndrome (SSS) and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.

**Morphology**

*Staphylococcus aureus* appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies often with hemolysis when grown on blood agar plates (Sherris, 2004).

**Signs and symptoms**

*Staphylococcus aureus* most commonly colonizes the anterior nares (the nostrils). The rest of the respiratory tract, open wounds, intravenous catheters and the urinary tract are also potential sites for infection. Healthy individuals may carry MRSA asymptotically for periods ranging from a few weeks to many years. Patients with compromised immune systems are at a significantly greater risk of symptomatic secondary infections.
In most patients, MRSA can be detected by swabbing the nostrils and isolating the bacteria found inside. MRSA may progress substantially within 24–48 hours of initial topical symptoms. After 72 hours, MRSA can take hold in human tissues and eventually become resistant to treatment. The initial presentation of MRSA are small red bumps that resemble pimples, spider bites or boils they may be accompanied by fever and occasionally rashes. Within a few days, the bumps become larger and more painful they eventually open into deep, pus-filled boils. About 75 percent of Community-Associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) infections are localized to skin and soft tissue which usually can be treated effectively but some CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional healthcare-associated HA-MRSA infections. They can affect vital organs and lead to widespread infections like sepsis, toxic shock syndrome and necrotizing pneumonia can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infections have been reported worldwide (Chambers *et al*., 2009).

MRSA is caused by strains of bacteria that have developed resistance to a number of widely used antibiotics. Although *Staphylococcus aureus* infections were historically treatable with common
antibiotics, emergence of drug-resistant organisms is now a major concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s and is now prevalent worldwide (DeLeo & Chambers, 2009; Liebowitz, 2009).

The frequency of MRSA infections continues to grow in hospital-associated settings, and more recently, in community settings globally. The increase in the incidence of infections due to *Staphylococcus aureus* is partially a consequence of advances in patient care and also of the pathogen’s ability to adapt to a changing environment. *Staphylococcus aureus* infections impose due to a high and increasing burden on health care resources. A growing concern is the emergence of MRSA infections in patients with no apparent risk factors (Boucher & Corey, 2008). The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999 (Verma et al., 2000). MRSA in tonsils may serve as a potential source for the spread of these organisms to other body sites as well to other individuals (Brook & Foote, 2006). MRSA is prevalent in many hospitals and often reflects the difficulties in hospitals and the health service generally, in terms of the control and prevention of healthcare-associated infection (Humphreys,
Multidrug-resistant bacteria, such as MRSA are endemic in healthcare settings in the United States and many other countries of the world. Nosocomial transmission of MRSA serves as a source of hospital outbreaks and recent reports of vancomycin resistant Staphylococcus aureus strains in the United States, emphasize the need for better control of MRSA and other resistant bacteria within healthcare settings (Henderson, 2006). MRSA bacteria are usually spread through skin-to-skin contact with someone who has an MRSA infection (Duerden, 2011). MRSA infections usually develop in people being treated in hospital, particularly patients in intensive care units (ICUs) and in surgical wards.

The treatment of infections due to Staphylococcus aureus was revolutionized in the 1940s by the introduction of the antibiotic penicillin (Johnson, 2007). However, most strains of Staphylococcus aureus are now resistant to penicillin. This is because Staphylococcus aureus can make a substance called β-lactamase that degrades penicillin, destroying its antibacterial activity.

In the early 1960s, a new type of penicillin antibiotic called methicillin was developed. Methicillin was not degraded by β-lactamase and so could be used to treat infections due to β-lactamase-producing strains of Staphylococcus aureus. Subsequently, methicillin was replaced by newer
and better penicillin-type antibiotics (such as flucloxacillin) that were also not affected by β-lactamase. Unfortunately, shortly after the introduction of methicillin, certain strains of *Staphylococcus aureus* emerged that were resistant to methicillin and also to the newer drugs such as flucloxacillin. These Methicillin Resistant *Staphylococcus aureus* came to be known as “MRSA” although methicillin is no longer prescribed (Johnson, 2007).

Although other types of antibiotics can still be used to treat infections caused by MRSA, these alternative drugs are mostly not available in tablet form and must be administered through a drip inserted into a vein or by injection. MRSA infections are a particular problem in hospitals. Some patients are at increased risk of developing infection. They include those with breaks in their skin due to wounds or indwelling catheters which allow MRSA to enter the body and those with certain types of deficiency in their immune system, such as low numbers of white cells in their blood. Individuals colonised with MRSA may also serve as a ‘reservoir’ of MRSA that may spread to other patients.

Some strains of MRSA are particularly successful at spreading between patients and may also spread between hospitals, presumably when colonized patients or staff, move from one hospital to another. These strains are known as epidemic MRSA or EMRSA. During the 1990s there
was a marked increase in infections caused by MRSA in hospitals in the UK due to the emergence spread of two particular strains of EMRSA known as EMRSA-15 and EMRSA-16 (Johnson, 2007).

To diagnose MRSA infection, specimens such as a swab of an infected wound or a sample of blood are taken from the patient. These are sent to a microbiology laboratory, where bacteria present in the specimen are cultured and identified. In addition, sometimes more rapid tests which detect the DNA typically found in MRSA may be undertaken. Colonisation with MRSA is detected similarly using swabs of a person's skin or from the inside of the nose.

**Some of the populations at risk in developing MRSA infections.**

People with weak immune systems (HIV/AIDS, lupus, cancer patients, transplant recipients, severe asthmatics, etc.), diabetics, intravenous drug users, users of quinolone antibiotics, young children, the elderly, college students living in dormitories, people staying or working in a health care facility for an extended period of time, urban under-served, people who spend time in confined spaces with other people, including occupants of homeless shelters and warming centers, prison inmates, military recruits in basic training (Zinderman et al., 2004) and individuals
who spend considerable time in change rooms or gyms (Tacconelli, 2008; Lipsky et al., 2010; David, 2010).

**Mechanisms of antibiotic resistance**

Three known mechanisms, account for the resistance of *Staphylococcus aureus* to the penicillin: hyper production of β-lactamases, modification of the normal penicillin-binding proteins (PBPs), and the presence of an acquired penicillin-binding protein (PBP2a) (Palavecino, 2007). Most clinical isolates demonstrate the latter. With this mechanism, when penicillin is bound to normal PBPs, *Staphylococcus aureus* strains are unable to properly assemble the cell wall, resulting in lysis and cell death. The unique, inducible, acquired PBP2a proteins produced by MRSA retain some low affinity for β-lactam antibiotics, although they permit antibiotic resistance in the presence of continued biosynthetic function (Chambers, 1997). Traditionally, because of the universal resistance of MRSA to β-lactams and because of the lack of other effective alternatives, the glycopeptide vancomycin became the mainstay of treatment, because it provides in-vitro activity against all staphylococci and demonstrates clinical response against MRSA infection (Fasola & Peterson, 1992).

Vancomycin was first isolated in 1953 at Eli Lilly from a soil sample collected from the interior jungles of Borneo by a missionary. It is a
naturally occurring antibiotic made by the soil bacterium Actinobacteria species *Amycolatopsis orientalis* (formerly *Nocardia orientalis*). The compound was industrially produced by fermentation and given the generic name vancomycin, derived from the term "vanquish". The original indication for vancomycin was for the treatment of penicillin-resistant *Staphylococcus aureus*. For many years since its initial use, vancomycin has traditionally been reserved as a drug of "last resort", used only after treatment with other antibiotics had failed. Recently, however, vancomycin resistant organisms are becoming common. Thus, vancomycin is increasingly being displaced from this role by newer antibiotics such as linezolid (Zyvox), daptomycin (Cubicin), and quinupristin/dalfopristin (Synercid). Teicoplanin is an antibiotic used in the prophylaxis and treatment of serious infections caused by Gram-positive bacteria, including Methicillin-Resistant *Staphylococcus aureus*. It is a semi-synthetic glycopeptide antibiotic. Its mechanism of action is to inhibit bacterial cell wall synthesis. Teicoplanin is marketed by Sanofi-Aventis under the trade name Targocid. Its strength is considered to be due to the length of the hydrocarbon chain (Gilpin & Milner, 2006).
Mechanisms of bacterial resistance to antimicrobial agents

The mechanisms behind the development and spread of bacterial resistance to antimicrobial drugs are reviewed. The chief mechanisms by which antimicrobials act are interference with nucleic acid synthesis, binding to ribosomes, and inhibition of cell-wall synthesis and folate metabolism. Bacteria have evolved genetic and biochemical ways of resisting these antimicrobial actions.

Genetic mechanisms include mutation and acquisition of new DNA. Bacteria resist antimicrobials biochemically by

- Inactivating the drugs with beta-lactamases, acetylases, adenylases, and phosphorylases; reducing drug access sites of action by virtue of membrane characteristics;
- Altering the drug target so that the antimicrobial no longer binds to it;
- Bypassing the drug's metabolism and developing tolerance.

Staphylococcal resistance to penicillin is expressed as beta-lactamase production, secretion of novel beta-lactamases, expression of novel penicillin-binding proteins (PBPs) to which penicillin bind poorly and increased production of or altered affinity to existing PBPs.

Glycopeptide resistance can be transferred clinically from enterococci to staphylococci. Vancomycin use is discouraged to limit the spread of
glycopeptide resistance. Many mechanisms are responsible for the development and spread of antimicrobial resistance. Resistance in *Staphylococcus aureus* first emerged in the form of strains with elevated Minimum Inhibitory Concentration (MIC) values towards vancomycin (or both vancomycin and teicoplanin), which have been named vancomycin-intermediate *Staphylococcus aureus* (VISA) or Glycopeptide-intermediate *Staphylococcus aureus* (GISA). Originally described in Japan in 1996 in Methicillin-resistant *Staphylococcus aureus* (MRSA) (Ino, 1997). VISA and GISA strains have now been isolated in numerous countries, particularly from patients having received prolonged vancomycin therapy (Hiramatsu, 2001; Hamilton, 2002). These bacteria are characterized by a thickened cell wall, production of an abundant extracellular material of still ill-characterized nature and an impaired ability to divide (Miller, 1999). These phenotypic changes can be explained by the production of an altered peptidoglycan with an increased proportion of free D-Ala-D-Ala termini (less reticulation) which can trap vancomycin molecules and prevent their access to the target at the cytosolic membrane. The thickened cell wall of a VISA strain may contain up to two to four times more D-Ala-D-Ala residues than that of a susceptible strain and is able to bind up to three to six times more vancomycin molecules before peptidoglycan synthesis become impaired.
(Hiramatsu, 2001). This mechanism also implies a reduced cross-linkage of peptidoglycan. The latter has been suggested to result from decreased activity of penicillin-binding proteins (PBPs) (Sieradzki & Tomasz, 1999) or from an alteration of murein precursors (Cui et al, 2000). Yet multiple, additive, but not clearly identified mutations are probably necessary to obtain resistance. VISA and GISA also need to import a larger amount of precursors than normal strains, which compromises their fitness in an antibiotic-free environment. This explains why VISA and GISA tend to lose their resistance when relieved from vancomycin pressure, giving rise to the so called hetero-VISA phenotype (Hiramatsu, 2002). In a still more frightening fashion, two MRSA strains with high levels of resistance to vancomycin and teicoplanin have now been reported in two different hospital institutions in the US (Mortal, 2002).

Vancomycin was first approved by the Food and Drug Administration in 1958, and resistance first emerged in coagulase-negative staphylococci in 1987 (Schwalbe et al., 1987). In 1996, the first clinical isolate of *Staphylococcus aureus* with reduced susceptibility to vancomycin was identified in Japan (Vancomycin-intermediate *Staphylococcus aureus* (VISA) (Hiramatsu, 1997). In July 1997 the Center for Disease Control and Prevention (CDC) issued an interim recommendation regarding prevention
and control of these strains (Garrett et al., 1997). In June 2002, a strain of *Staphylococcus aureus* fully resistant to vancomycin (Vancomycin-resistance *Staphylococcus aureus* (VRSA) was isolated from a patient in Michigan (Sievert et al., 2002). Conjugate transfer for the vanA gene from enterococci to *Staphylococcus aureus* had previously been demonstrated *in vitro* (Noble et al., 1992). Among enterococci, four phenotypes of glycopeptide resistance have been reported in the literature: vanA phenotype with high-level resistance to vancomycin and teicoplanin, vanB phenotype with resistance to vancomycin only, vanC phenotype and a “vanC-like” phenotype (Clark et al., 1993).

**Vancomycin Intermediate Staphylococcus aureus (VISA)**

VISA was first identified in Japan in 1996 and has since been found in hospitals elsewhere in Asia, as well as in the United Kingdom, France, the U.S. and Brazil. It is also termed GISA (Glycopeptide Intermediate *Staphylococcus aureus*), indicating resistance to all glycopeptide antibiotics. These bacterial strains present a thickening of the cell wall, which is believed to reduce the ability of vancomycin to diffuse into the division septum of the cell required for effective vancomycin treatment.

Even with the absence of high-level resistance to vancomycin, another concern is proposed by the presence of VISA is the increased
difficulty in prescribing treatments, especially in situations where an effective treatment for an infection is needed urgently, before detailed resistance profiles can be obtained. In hospitals already endemic with multi-resistant MRSA, the appearance of VRSA would make the treatment of infected patients much more difficult.

**Vancomycin-resistant *Staphylococcus aureus* (VRSA)**

High-level vancomycin resistance in *Staphylococcus aureus* has been rarely reported. *In vitro* and *in vivo* experiments reported in 1992, demonstrated that vancomycin resistance genes from *Enterococcus faecalis* could be transferred by horizontal gene transfer to *Staphylococcus aureus*, conferring high-level vancomycin resistance to *Staphylococcus aureus*. Until 2002 such a genetic transfer was not reported for wild *Staphylococcus aureus* strains. In 2002, a VRSA strain was isolated from the catheter tip of a diabetic, renal dialysis patient in Michigan. The isolate contained the mecA gene for methicillin resistance. Vancomycin MICs of the VRSA isolate were consistent with the VanA phenotype of enterococcus species, and the presence of the vanA gene was confirmed by polymerase chain reaction. The DNA sequence of the VRSA vanA gene was identical to that of a vancomycin-resistant strain of *Enterococcus faecalis* recovered from the same catheter tip. From 2002 to 2010, ten
additional VRSA isolates were reported, eight from the United States, one from Iran, and one from India.

**Patients infected with MRSA**

Patients with infections due to *Staphylococcus aureus* often need antibiotics. Infections due to normal strains of *Staphylococcus aureus* are often treated with flucloxacillin (eg. Floxapen), but this is ineffective against MRSA. To make matters worse, MRSA are often also resistant to other types of antibiotics such as erythromycin and ciprofloxacin (Allan, 2007).

Although MRSA are resistant to many drugs, most remain susceptible to the antibiotics vancomycin and teicoplanin. Infections due to MRSA are therefore often treated with one or other of these drugs. Both must be administered by infusion or injection and for this reason, they are used for treatment only in hospitalised patients. Injection of vancomycin into muscle is painful and thus not used, while rapid administration into a vein may produce an allergic-type reaction ('red man' syndrome). To overcome these problems, vancomycin must be given by slow infusion into a vein. In contrast, teicoplanin may be safely administered by injection into muscle or rapid infusion into a vein.

A very few MRSA resistant to vancomycin and/or teicoplanin have been found in the USA and although there is concern that they may
become more common, there is no evidence of this happening to date. Fortunately, in the last few years further antibiotics that are active against MRSA have been developed and licensed for clinical use. One such drug, called linezolid may be given either by intravenous infusion (in severely ill patients) or in tablet form. Clinical trials have so far shown it is useful (either alone or in combination with other antibiotics) for the treatment of pneumonia and skin and soft tissue infections. More recently, another drug called daptomycin has been licensed for the treatment of skin, soft tissue, heart and blood infections including those caused by MRSA (Sakoulas, 2008)

The primary aim of this study is to observe the susceptibility pattern of Glycopeptide resistance in Methicillin Resistant Staphylococcus aureus (MRSA) isolates. For this various samples such as pus swabs, wound swabs, fluids like ascitic and synovial fluids and blood culture’s were obtained. Antibiogram was placed and epsilometer test was performed for vancomycin and teicoplanin which are glycopeptides. Collectively these results were used for evaluation of glycopeptide resistance in MRSA.
OBJECTIVES OF THE PRESENT STUDY

1. To observe the susceptibility of Glycopeptide resistance in Methicillin Resistant *Staphylococcus aureus* (MRSA) by determining the Minimum Inhibitory Concentration (MIC) through E-test

2. To recommend administration of right antibiotic to the patient who is suffering from MRSA infection.