Chapter-II

Review Of Literature
2. REVIEW OF LITERATURE

2.1. STAPHYLOCOCCUS AUREUS

The organism first developed by Alexander Ogston over a hundred years ago, has proved its might as the most versatile pathogen. It has maintained its role as one of the commonest pathogens in both community acquired and hospital acquired infections.

Robert Koch was the first to observe these spherical microorganisms in pus and to incriminate them as etiological agents for suppurative lesions. Both Pasteur (1880) and Ogston (1881) further substantiated Koch’s postulate on the production of specific disease by specific microorganisms with respect to abscess formation. During, the later half of the 19th century, the presence of spherical microorganisms in the suppurative lesion had been repeatedly observed under the microscope. The name ‘Staphylococcus’ for cocci arranged in clusters was derived from the Greek word ‘Staphyle’ meaning a bunch of grapes. Even though Ogston (1881) originally proposed the name, Rosenbach (1884) was the first to adopt the name Staphylococcus as the generic name. He divided them into 2 species, namely Staphylococcus aureus (golden yellow) is a bacterium, frequently living on the skin or in the nose of a healthy person, and Staphylococcus albus (white) based on colonial pigmentation.
2.2. CLASSIFICATION

According to the ninth edition of Bergey’s Manual of Determinative Bacteriology, the systemic position of the genus *Staphylococcus* is as follows:

Kingdom :  Bacteria  
Phylum :  Firmicutes  
Class :  Bacilli  
Order :  Bacillales  
Family :  Staphylococcaceae  
Genus :  *Staphylococcus*  
Species :  *S. aureus*

The application of modern taxonomic concepts to the staphylococci can be said to have begun with the studies of Baird-Parker (1965). Many phenotypic characters of a large collection of staphylococci and micrococci were examined, and a judicious selection was made of those of most use for classification. The anaerobic glucose fermentation test was used to distinguish staphylococci from micrococci and 6 groups were defined within *Staphylococcus* by means of a number of biochemical and physiological characters. *Micrococcus* was divided into 8 subgroups. Nineteen species of *Staphylococcus* are recognized in the current edition of Bergey’s manual of systematic bacteriology (Kloos and Schleifer, 1986), a number that has now risen to 26 (Freney *et al.*, 1988). Species at present recognized in the genus *Staphylococcus* are clearly related to each other, as shown by comparative biochemical and nucleic acid pairing and sequencing data (Zakrzewska-Czerwinska *et al.*, 1988). The status of the
staphylococcal species is supported by evidence from DNA pairing, protein homology and numerical phenotypic studies (Goodfellow et al., 1987).

2.3. CHARACTERIZATION

2.3.1. Cultural

*S. aureus* grows readily on ordinary media with in a temperature range from 10 to 42ºC, the optimum temperature being 37ºC and pH 7.4 to 7.6. On nutrient agar, after incubation for 24 hrs, the colonies are large measuring 2 to 4 mm in diameter. The growth of *S. aureus* is butyrous and easily emulsifiable. Colonies are smooth, raised, glistening, circular, entire and translucent. With increasing age, colonies become nearly transparent on other media. Colonial pigment is variable, ranging from white with a yellowish tint to a characteristic golden yellow color (Marshall et al., 1981). Unpigmented cells contain very low quantities of carotenoid pigment. The production of pigment may be influenced by growth conditions; it can be intensified by growth on media containing 10% bovine serum (O’Conner et al., 1966). On mannitol salt agar, colonies appear yellow in colour due to the fermentation of mannitol. And on blood agar β-hemolysis occurs. They grow on Macconkey’s medium, producing smaller colonies that are pink due to lactose fermentation.

2.3.2. Microscopic
S. aureus is a gram positive, non-motile, asporogenous and capsulated bacterium that form spherical cells with an average diameter of 0.5 to 1.5 µm. The size varies from strain to strain and is also influenced by the age of the culture and by the medium on which it is grown. The cocci are arranged characteristically in grape like clusters due to cell division occurring in three planes; with daughter cells tending to remain in close proximity (Enright, 2002).

2.3.3. Biochemical characterization

S. aureus ferments a number of sugars producing acid but no gas. This is a diagnostic value except for mannitol, which is usually fermented aerobically. S. aureus produce β-type of haemolysis on blood agar, produce golden yellow colour on nutrient agar. They are catalase positive, coagulase positive, liquefy gelatin and most strains are lipolytic producing a dense opacity when grown on media containing egg yolk, production of phosphate can be demonstrated by culturing on nutrient agar containing phenolphthalein diphosphate. when a such a culture is exposed to ammonia vapour, colonies assume a bright pink colour due to the presence of free phenolphthalein.

2.4. STRUCTURE OF S. AUREUS

2.4.1. Genome

The genome of any organism is the product of countless generations of existence and life history of that organism (Hiramatsu et al., 2004). Knowledge of bacterial structures and functions has increased and will further increase owing to recent advances in genome sequencing and other genome-wide analysis. To date,
genomes of 7 *S. aureus* strains have been elucidated COL, NCTC 8325, N315, MU50, MW2, EMRA-16 and MSSA 476 (Baba et al., 2002; Kuroda et al., 2001).

*S. aureus* strain N315, an isolate of pharyngeal smear from an uninfected in-patient possess as many as 39 toxin genes and 100 other genes presumably involved in pathogenesis, which together constitutes 5.4% of the 2,595 protein-coding open reading frames (ORFs) on the chromosome (Steinmetz and Davis, 2000). Of all the ORFs found on the *S. aureus* chromosome, about 52% have their strongest homologies to the corresponding ORFs (orthologs) of *Bacillus* species. They are mostly essential genes for the vital processes of the organism, such as energy formation and multiplication.

*Staphylococcus aureus* is able to cause infections and spread from the prevailing infections around. The implanted medical devices often serve as sources of *Staphylococcus epidermidis*. Steven et al., (2004) have sequenced the ~2.8-Mb genome of *S. aureus* and performed comparative analysis with other staphylococcal genomes to find out the virulence factors in the genomes. It was found that genomic islands in nonsyntenic regions harbored variations leading to pathogenicity and resistance. Gene transfer events between staphylococci and low GC content gram positive bacteria seem to have played an important role in the evolution of virulence and resistant properties. It was also found that several genes for cadmium resistance and species specific LPXTG surface proteins were found in the plasmids in the *S. epidermis* (steven et al 2004).
S. aureus genome contains four ORFs that potentially encode proteins having similarity to human major histocompatibility complexes (MHC), indicating the possibility that S. aureus acquired the MHC genes through its close contact with human tissues in the distant past of its evolutionary timescale. 26 genes constituting two thirds of all the toxin genes are predicted to encode superantigens on S. aureus chromosome, indicating the importance of superantigens in the pathogenic potential of S. aureus (Hiramatsu et al., 2004).

Like virulence genes, antimicrobial resistance genes are exogenously introduced into S. aureus cells by lateral genetic transfer. The genetic vehicles responsible for the transfer of resistance genes seem to be different from those responsible for virulence gene transfer as noted in the Fig.2a. Virulence associated genes are found in various parts of the chromosome as either single genes or clusters on pathogenicity islands (PIs). The rest are in integrated copies of bacteriophages.

Staphylococcus aureus is a major cause of antimicrobial-resistant infections of humans. Hybrids of S. aureus, which originate from large-scale chromosomal recombinations between parents of distinct genetic backgrounds, are of interest from clinical and evolutionary perspectives (Jonathan et al 2012)

The antimicrobial resistance profile of each strain of MRSA, therefore, can be evaluated by detecting the resistance genes identified on SCCmec or plasmids. Rapid detection of resistance genes in the genome of a pathogen will shorten the time and labour required for the determination of the antimicrobial susceptibility profile of the
pathogen. This will enable early treatment of infections and thus improve the effectiveness of empiric therapy.

The strain is resistant to a wide range of antibiotics, antiseptics, and heavy metals due to resistance genes encoded on mobile genetic elements and also mutations in housekeeping genes (Matthew et al. 2009).

2.4.2. Cell wall

The outermost layers of pathogens are important in the infection process (Anthony and Hill, 1988). Most S. aureus isolates are covered by polysaccharide capsule. Beneath the capsule, S. aureus harbors a typical Gram positive cell wall (Giesbrecht et al., 1998). The Gram-positive cell wall has thicker and highly cross-linked peptidoglycan layer and it lacks the outer membrane (Beveridge, 1999; Popescu and Doyle, 1996; Van Wely et al., 2001) as shown in Fig. 2b. S. aureus produces four Penicillin Binding Proteins (PBP1-4), involved in cell wall peptidoglycan assembly (Labischinski, 1992). The biological activity of these native PBPs is similar to that of serineproteases, and they act as transpeptidases in the cross linking of the glycan chains (Murakami et al., 1994; Waxman and Strominger, 1983). PBP2 is a
bifunctional protein which, in addition to transpeptidase activity, also acts as transglycosylase (Goffin and Ghuysen, 1998). PBPs bind effectively to β-lactam antibiotics and in the presence of these agents the cell wall assembly is discontinued. Another common feature of *S. aureus* cell wall is teichoic acid, a carbohydrate-phosphate polymer covalently linked to MurNAC (Baddiley, 1989). Teichoic acids bind divalent cations and possess antigenic properties.

![Fig.2b: Schematic presentation of *S. aureus* cell wall](image)

**2.4.3. Capsule**

Most staphylococci produce microcapsules. Karakawa and Vann (1982) have described eleven capsular serotypes by typing with the polyclonal and later with monoclonal antibodies. Of the 11 types of microcapsular polysaccharide serotypes that have been identified, types 5 and 8 account for 75 percent of human infections.
Most methicillin-resistant *S. aureus* isolates are type 5. The chemical composition of four of these antiphagocytic polysaccharides, including types 5 and 8, has been determined, and all four have been shown to be chemically related (Lee, 1996).

### 2.4.4. Surface proteins

Many staphylococcal surface proteins have certain structural features in common. These features include a secretory signal sequence at the N terminal, positively charged amino acids that extend into the cytoplasm, a hydrophobic membrane-spanning domain, and a cell wall anchoring region, all at the carboxyl terminal. A ligand binding domain at the N terminal that is exposed on the surface of the bacterial cell enables some of these proteins to function as adhesions (Foster and McDevitt, 1994). Kaplan and Tenenbaum (1982) explained that Protein A, is immunologically active substance and has antiphagocytic properties that are based on its ability to bind the Fc portion of immunoglobulin.

Patti *et al.* (1994) reviewed extensively and explained that *S. aureus* colonizes the host to initiate infection by adhering to the components of the extracellular matrix. The adherence is mediated by surface proteins and have been designated microbial surface components recognizing adhesive matrix molecules (MSCRAMM).

### 2.5. CARRIAGE
Kloos and Schleifer (1981) and Kloos (1980) demonstrated that natural population of staphylococci are associated with skin and mucous membranes. The major habitat of staphylococci in man includes the anterior nares, axillae, perineum, toe webs, general skin surfaces, hands etc. Noble et al. (1964) and Zierdt (1982) have shown in their experiments that there is 10-40% carriage rate of *S. aureus* in the anterior nares of the adults that are not hospitalized. Noble and Somerville (1974) explained that the nasal carriage of *S. aureus* is comparatively less frequent in adults than in children.

Nasal carriage of *S. aureus* is one of the major risk factors for *S. aureus* infection (Klutymans et al., 1997). The primary source of *S. aureus* in humans is the anterior nasal tracts and nevertheless it can be found in different parts of the body (Doebbling, 1994; Reagen et al., 1991). Apparently the *Staphylococcus* flourish here in the relative absence of human defenses and capable of withstanding the local antibacterial defenses (Klutymans et al., 1997).

In the healthy populations 10 to 35% of individuals carry *S. aureus* persistently, 20 to 75% intermittently and 5 to 50% never carry *S. aureus* in the nose. Both methicillin resistant and methicillin sensitive isolates are persistant colonisers (Casewell and Hill, 1985; Stanford et al., 1994). Proportions of nasal carriage patterns differ, depending on the study design and definition for persistent, intermittent and non carriers (Nouwen et al., 2001). *S. aureus* infections is also of major concern for age, renal dialysis, repeated injury to the skin, liver diseases and diabetes.
2.6. PATHOGENESIS

The five stages in the pathogenesis of *S. aureus* infections are (1) colonization, (2) local infection, (3) systemic dissemination and/or sepsis, (4) metastatic infection and (5) toxinosis. Approximately 30% of the healthy individuals are colonized by *S. aureus*, usually in the anterior nares. *S. aureus* can be present without causing infections for weeks and months on mucous membranes but on skin it can stay for quite less time. Colonization precedes infection. Local abscesses of skin result when the organism is inoculated into the skin from a site of carriage. The infection can spread locally or can gain access to the blood (Gordon, 1998).

Once in the blood, the organism spreads widely to peripheral sites in distant organs, and septic shock can ensue. As a result of hematogenous dissemination, a number of staphylococcal infections can result. Finally, even if the organism itself does not invade the bloodstream, specific syndromes can result from the local or systemic effects of specific toxins (Matsushashi *et al.*, 1986).

2.7. VIRULENCE FACTORS
The extensive virulence components possessed by S. aureus makes it a successful pathogen and therefore can cause varied infections. It displays a wider variety of virulence mechanisms than virtually any other human pathogen and is a model for the study of the pathogenesis of infectious diseases (Projan and Novick, 1997). A list of those factors known to contribute to the virulence of the organism is shown in Table-2a.

Specific factors are present that allow the organism to initially thwart opsonophagocytosis, walling off the infection to form an abscess; to invade through tissue from the initial site of infection; to induce the sepsis syndrome by promoting massive cytokine release; to move out of the blood into underlying tissue by attaching to and invading endothelial cells and to produce specific syndromes by toxin production. There are also specific global regulatory systems such as agr and sar, that determine which virulence factors are produced at specific times during growth and in response to the local environment (Projan and Novick, 1997).

The pathogenicity of S. aureus is caused by several enzymes and toxins which are as follows.

2.7.1. Enzymes
Catalase: All *Staphylococcus* species produce catalase. This enzyme breaks down hydrogen peroxide into water and oxygen. Hydrogen peroxide is used by neutrophils during phagocytosis. The neutrophils use hydrogen peroxide to form toxic oxygen radicals. The presence of catalase counteracts this mechanism (Talaro and Talaro, 1996).

Coagulase: *S. aureus* and *S. intermedius* strains produce coagulase. This enzyme is a prothrombin activator, converts fibrinogen to fibrin. Its contribution to bacterial virulence is uncertain (Franklin and Lowy, 1998).

Hyaluronidase: This enzyme is also known as the spreading factor. It digests the intracellular glue or hyaluronic acid that binds the connective tissue in the host (Talaro and Talaro, 1996).

β-lactamase: This extracellular enzyme opens the β-lactam ring of penicillin based antibiotics. A penicillin binding protein is responsible for staphylococci resistant to the penicillins and cephalosporins (Wilkinson, 1997).

2.7.2. Toxins
**Hemolysins:** This group of toxins causes the lysis of red blood cells. There are four different types of this toxin $\alpha$, $\beta$, $\gamma$ and $\delta$. $\alpha$-haemolysin is dermonecrotic, neurotoxic, andlyses red blood cells (Bhakdi and Tranum-Jenson, 1991). It also damages leukocytes, and skeletal muscle, and heart and renal tissue. $\beta$-haemolysin acts as sphingomyelinase. $\delta$-haemolysin has leucocytolytic activity. $\gamma$-haemolysins act as a detergent disrupting biologic membranes (Dinges *et al.*, 2000).

**Exotoxins:** The two most important exotoxins are leukocidin and enterotoxin. Leukocidin is a multicomponent two fractions (S and F) protein toxin produced as separate damages the cell membrane of macrophages and neutrophils and is another way to inhibit the phagocytic host defense. Enterotoxins act on the gastrointestinal tract of humans to produce diarrhea (Talaro and Talaro, 1996).

**Exfoliative toxin:** This toxin separates the epidermal layer of the skin from the dermis causing it to peel away and is responsible for staphylococcal scalded skin syndrome. Exfoliative toxins TSST 1 and enterotoxins A-E and G-J are potent superantigens (Ladhani *et al.*, 1999).

**CLINICAL MANIFESTATIONS:**

*Staphylococcus aureus* causes wide variety of diseases, from mild skin infections to severe life threatening systemic infections (Waldvogel, 2000). Infections caused by *S. aureus* can either be localized or systemic. This depends on the degree of
invasion and toxin production by the bacteria. Localized infections are commonly known as abscesses. Systemic infections include bacteremia, endocarditis, osteomyelitis, and pneumonia. There are also two toxigenic staphylococcal diseases: toxic shock syndrome and staphylococcal scalded skin syndrome.

2.8.1. Abscesses

*Staphylococci* may invade the skin in several ways including wounds, follicles, or skin glands. The most common type of infection is folliculitis. These infections are a mild, superficial inflammation of hair follicles or glands. These infections are usually self-limiting but may progress to subcutaneous tissue infections. Furuncles or boils are a progression of folliculitis to a large red tender pustule. These often occur in clusters, in friction bearing areas of the body such as buttocks, breasts, axillae and back of the neck. Carbuncles are an aggregation of a cluster of furuncles. These infections are much larger and more painful. They usually appear on areas of thicker skin such as the back of the neck. This infection may progress to systemic disease. Another type of staphylococcal skin infection is impetigo. This infection is characterized by bubble-like epidermal swellings and may break and peel away (Kenneth and Ryan, 1994).

The genes responsible for the bacterial persistence and abscess formation during *S. aureus* infections are yet to be discovered. We show here that following intravenous infection of mice, *S. aureus* disseminates very quickly into different organs and start developing lesions that grow over weeks but cannot be prevented by the host. (Alice et al 2009)
2.8.2. Bacteremia

The frequency of complications from staphylococcal bacteremia is high, ranging from 11 to 53%. An increasing percentage of bacteremic infections are related to catheterization (Steinberg et al., 1996). The incidence of this infection has dramatically increased in the last decades, occurring in both community acquired and hospital acquired cases. The predominant factor associated with this trend appears to be the growing use of intravascular devices.

Use of injecting drugs is another contributing factor to the increased incidence of *S. aureus* bacteremia (Blot, 2002). Users of injecting drugs often have nasal colonization with *S. aureus* and likely transmit the organism to the skin and eventually to the bloodstream. Factors associated with increased mortality due to bacteremia are over 50 years of age, non-removable foci of infection, and serious underlying cardiac, neurologic, or respiratory disease (Franklin and Lowy, 1998).

2.8.3. Endocarditis

Endocarditis is the inflammation of the valves and lining of the heart. The incidence of endocarditis caused by *S. aureus* may account for 25-35% of all endocarditis cases. Some risk factors for staphylococcal endocarditis are being an intravenous drug user, elderly patients, patients with prosthetic valves, and hospitalized
patients. *S. aureus* endocarditis differs in presentation from other endocarditis by its rapid onset, high fever, and frequent involvement of normal cardiac valves (Franklin and Lowy, 1998). The patients also tend to be younger and have a lower mortality rate. *S. aureus* is one of the most common pathogens in nosocomial and prosthetic valve endocarditis. Intravenous catheters are the most frequent source of bacterial inoculation.

### 2.8.4. Osteomyelitis

Osteomyelitis is an infection of the vascular metaphysis of bones. The bones that are most commonly involved are the femur, tibia, ankle, or wrist. Necrosis of bony tissue and abscess formation lead to an elevated and tender lump. Osteomyelitis occurs in two forms, primary and secondary. Primary osteomyelitis is typically seen in growing children, adolescents, and intravenous drug users. Secondary or traumatic osteomyelitis typically develops after a compound fracture or surgery in cancer or diabetes patients (Talaro and Talaro, 1996).

### 2.8.5. Pneumonia

*S. aureus* can be aspirated into the lungs and cause pneumonia as the bacterium frequently colonizes the nasopharynx. The fatality rate is 50%, even though *S. aureus* accounts for only a very small proportion of pneumonia cases. In community-acquired cases, isolation of *S. aureus* in sputum specimen most often represents contamination of the specimen by flora in the upper airway (Francis, 2005).
2.8.6. Toxic Shock Syndrome

Toxic shock syndrome came into prominence when numerous cases were associated with the introduction of super absorbent tampons. The disease had a fulminant onset and was often seen in previously healthy females. Toxic shock syndrome often develops from a site of colonization rather than infection (Chesney et al., 1981). Although toxic shock syndrome toxin-I is present in 90% of toxic shock syndrome menstrual cases, however other toxic shock syndrome toxins have been associated with non-menstrual cases. Patients with non-menstrual toxic shock syndrome have a higher mortality rate than those associated with menstruation (Wergeland et al., 1989).

2.8.7. Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome is commonly seen in children below five years. Scalded skin syndrome originates with a localized skin infection (Vogelaers, 2008). These infections lead to toxemia and when the toxin reaches the skin it induces a painful bright red flush over the entire body. The skin then blisters followed, by desquamation of the epidermis. The majority of cases have been described in infants and children under age four. Exfoliative toxin is responsible for this infection. This toxin also causes Staphylococcal impetigo which can affect all ages (Ladhani et al., 1999).
2.8.8. Wounds and Burns

*Staphylococcus aureus* is a major cause of wound infections. The source may be clinical or carrier state. Surgical wound infections can be very severe and infections at the site of intravenous lines can result in bacteremia and metastatic infection. *S. aureus* is the dominant species in surgical wound infections (Meers et al., 1981). Most infections of surgical wounds occur at the time of operation. Staphylococcal post surgical infections are characterized by progressive appearance edema, erythema and pain around the surgical incision two or more days after surgery.

*S. aureus* is responsible for causing most of the secondary infections in most burn cases. These provide a suitable site for bacterial multiplication. When this has taken place, the burn is richer and more persistent source of infection than the surgical wound because a large area of tissue is exposed for a longer time. The clinical consequences of infection in burns may be very serious; a large proportion of the mortality in burn patients who survive the initial trauma and shock is due to infection (Pruitt, 1984).

2.9. TREATMENT

Emerging resistance in MRSA strains makes infections extremely difficult to treat. Treatment options are limited to the groups of new quinolones, oxazolidinones, quinupristin-dalfopristin, various combinations of antibiotics, and new investigational compounds. Unfortunately, none of these agents has been clinically tested on a
sufficient scale. Methicillin-susceptible *S. aureus* is typically treated with a β-Lactam antibiotic, such as flucloxacillin or cloxacillin. For the treatment of superficial infections caused by *S. aureus*, amoxicillin, erythromycin, co-amoxiclav and topical fusidic acid or mupirocin is used (Wenzel and Perl, 1995). Clindamycin has been used widely in the treatment of skin and soft tissue infections. For the treatment of staphylococcal infections, antimicrobial agents given intravenously are more effective in combating the disease. Vancomycin remains a first line therapy for severe infections possibly caused by MRSA. Other intravenous agents such as clindamycin, daptomycin, linezolid, quinopristin-dalfopristin may be appropriate (Franklin and Lowy, 2003).

### 2.10. ANTIBIOTIC RESISTANCE

The appearance of antibiotic resistant staphylococci over the past 40 years has been regarded as an inevitable genetic response to the selective pressure imposed by antimicrobial therapy. With the increased virulence capacity, more diversified infections, and its ability to adapt to different conditions makes *S. aureus* the pathogen of highest concern (Waldvogel, 2000 the major causative agent for the nosocomial infections is *S. aureus* and it is posing increased concerns in the community being treated. (Diekema *et al.*, 2001). Due to the increasing problems faced by *S. aureus*, antibiotics were used for its treatment since the antibiotic period. But it again responded by developing resistance to the antibiotics used by: (1) mutating the genes responsible for the susceptibility of the *S. aureus* and selecting resistant strains and (2) acquiring resistance genes from other organisms as extra chromosomal plasmids, and transposons. (Tomasz, 1994).
The mortality of patients with *S. aureus* (Charbonneau, 2006) bacteremia in the pre-antibiotic era exceeded 80%. Since the introduction of penicillin, *S. aureus* started acquiring resistance to the introduced antibiotics. (Rammelkamp and Maxon, 1942). By the late 1960s, more than 80% of both community and hospital acquired staphylococcal isolates were resistant to penicillin. This pattern of resistance, first emerging in hospitals and then spreading to the community, is now, a well established pattern that recurs with each new wave of antimicrobial resistance (Chambers, 2001).

*S. aureus* is responsible for the community based infections from both rural and urban societies. Great concern is of the few communities which possess MRSA infections (Moreno *et al.*, 1995; Herold *et al.*, 1998; Groom *et al.*, 2008).

### 2.10.1. *mecA*

Methicillin resistance is primarily caused by the *mecA* gene encoding a 78-kDa penicillin-binding protein 2A (PBP2A or PBP 2’) (Matsuhashi *et al.*, 1986 and Ubukata *et al.*, 1985). *S. aureus* has four native PBPs, which are enzymes catalyzing transpeptidase and transglycosidase reactions during peptidoglycan synthesis.

β-lactam antibiotics quickly acylate these enzymes because of their structural similarity with the natural substrates of the PBPs. The affinity of these antibiotics towards PBP2A is, however, much lower and this enzyme remains functional. Actually,
a partially functional native PBP in addition to PBP2A is needed in the presence of β-lactam antibiotics. The transglycosylase domain of the native PBP2 and the transpeptidase domain of PBP2A cooperate in cell wall synthesis of MRSA (Pinho et al., 2006).

\[ \textit{mecA} \text{ is preceded by the regulatory genes } \textit{mecR1} \text{ and } \textit{mecl}, \text{ which are transcribed to the opposite direction as compared to } \textit{mecA} \text{ (Hiramatsu et al., 1992 and Tesch et al., 1990). Based on homology to the plasmid-encoded penicillinase regulators } \textit{BlaR1} \text{ and } \textit{BlaI}, \text{ which in addition to regulation of penicillinase production are also able to regulate PBP2A production, } \textit{mecR1} \text{ is presumed to act as a signal transducer sensing the extracellular β-lactam antibiotic. This leads to activation of its cytoplasmic protease domain and specific cleavage of } \textit{mecl}, \text{ the transcriptional repressor of } \textit{mecA}. \text{ Most of the clinical MRSA isolates have deletions or point mutations in the regulatory area and they constitutively produce PBP2A. Intact regulator genes strongly repress PBP2A production, and it is poorly inducible even in the presence of many β-lactam antibiotics, including methicillin.}

\[ \text{It is believed that MRSA has evolved from MSSA by the acquisition of large genetic element known as SCCmec. SCCmec is a gene complex comprising of resistance genes against non β-lactam antibiotics..} \]

\textbf{2.10.2. SCCmec}
mecA is a part of mobile genetic element found in all MRSA strains. Katayama et al. (2000) demonstrated that mecA is a part of genomic island designated staphylococcal cassette chromosome mec (SCCmec) region. SCCmec is inserted into S. aureus chromosome near to the origin of replication at a well conserved attBscC site in the orfX gene of unknown function. The 15 bp att sequence is also present in the SCCmec region. After integration this sequence is found as direct repeats at both ends of SCCmec. In addition, degenerate inverted repeats are found at the ends of the SCCmec (Ito et al., 1999).

Five types of SCCmec have been characterized so far (Fig.2c) (Ito et al., 1999; 2001; 2004). Each type contains a mec gene complex with the resistance encoding mecA gene and intact or partly deleted regulatory genes. The region also contains a cassette chromosome recombinase (ccr) gene complex encoding site-specific recombinases of the invertase/resolvase family, which are responsible for integration and excision of SCCmec, as well as additional type specific DNA. The characteristics of different types of SCCmec are as shown in Table-2b and Fig.2c.

**Type I SCCmec** is 34 kb in length and possesses a class B mec complex: includes IS431-mecA-ΔmecR1-IS1272, and the ccr genes A1 and B1.

**Type II SCCmec** contains a class A mec complex: IS431-mecA-mecR1-mecI, and ccr genes A2 and B2.
**Type III SCCmec** has the class A *mec* complex with *ccrA3* and *B3*.

**Type IV SCCmec** has the class B *mec* complex with *ccrA2* and *B2* genes.

The recently found **Type V SCCmec** has a class C2 *mec* complex: IS431-*mecA*-$\Delta$mecR1-IS431, and a single *ccr* gene designated *C*. The enzymes encoded by *ccrA* and *B* genes together, and by *ccrC* on its own are able to catalyze the precise excision and integration of the SCCmec element (Ito *et al.*, 2004 and Katayama *et al.*, 2000).

*S. aureus* occurs in both hospital and community settings; the following table depicts the origin of different types of SCCmec:

Type I, IV and V SCCmec elements carry no other resistance genes in addition to *mecA*. Type II and III SCCmec elements carry resistance for spectinomycin and erythromycin encoded by Tn554. In addition, resistance for tobramycin, kanamycin and bleomycin, encoded by pUB110, are carried by type II SCCmec. $\Psi$Tn554 codes for cadmium resistance and pT181 for tetracycline resistance. Both of these as well as mercury resistance are carried by type III SCCmec. Type II and III elements contain several copies of IS431 sequences in addition to those associated with the *mec* complex. These insertion sequences flank the plasmids integrated in SCCmec. *pls* gene is a part of type I element, where as the type II element carries genes homologous to *kdp* operon encoding ATP-dependent potassium transport across the
bacterial membrane. Type V element carries *hsd* genes encoding for restriction modification system.

All these findings have led to the idea that SCC*mec* elements serve as vehicles for transfer of genetic information between staphylococcal strains and species.

2.11. EVOLUTION OF MRSA

First MRSA strains were described in 1961, at the same time period when methicillin was introduced into clinical use (Jevons, 1961) and since then has gradually disseminated, reaching epidemic proportions in some European countries in the 1960s and in the USA in the 1970s (Haley *et al.*, 1982). The origins of the *mecA* gene are still unclear. A *mecA* homologue with a close sequence similarity (overall deduced amino acid similarity of 88%) to the *mecA* of *S. aureus* (Wu *et al.*, 1996) was identified in isolates of *Staphylococcus sciuri* (Couto *et al.*, 1996). These isolates were found to be uniformly susceptible to β-lactam antibiotics; the function of this native gene of *S. sciuri* was not elucidated. However, when *S. sciuri* mutants were grown in presence of increasing methicillin concentrations, they accumulated increasing amounts of *mecA* transcripts due to a mutation in the promoter region of its gene. And hence it was successful in conferring resistance in *S. aureus* strain as well. It was shown that *S. sciuri* mutants selected in the presence of increasing concentrations of methicillin showed a drastic increase in the transcription rate of the *mecA* homologue, due to a point mutation in the promoter, and this mutated gene was able to confer increased resistance when introduced into a methicillin-susceptible *S. aureus* strain (Wu *et al.*, 1996).
2009). These results indicate that the *meca* homologue ubiquitous in the antibiotic-susceptible animal species *S. sciuri*, may be an evolutionary precursor of the *meca* of the MRSA strains.

It would be very surprising to know that recently a strain of MRSA was reported to be isolated from a neonate who never had any MRSA infection. (Wielders *et al.*, 2007). And this MRSA possessed *meca* genetic element from the *S. epidermidis* strain from the same patient but not similar to other MRSA strains. These results suggest that this particular MRSA was formed *in vivo* by horizontal transfer of the *meca* DNA between two *Staphylococcus* species.

2.12. MRSA IN HOSPITALS (HA-MRSA)

*Staphylococcus aureus* is one of the major causative agents of nosocomial bacteremia (Edmond *et al.*, 1999), postoperative wound infection (Nichols, 1998) and catheter related infections (Elliott and Faroqui, 1992). Most nosocomial *S. aureus* infections are endogenous, caused by the patient’s own carriage strain (Kropec *et al.*, 1993). Prevalence studies show that MRSA is a major cause of nosocomial infections worldwide, and may account for up to 20-40% of all *S. aureus* infections (Voss *et al.*, 1994). The proportion of methicillin resistant *S. aureus* varies among hospitals of individual countries. Extensive use of antibiotics within a hospital may partly explain differences among hospitals in transmission rates of resistant organisms (Shopsin *et al.*, 2005).
The main reservoir of MRSA in hospitals consists of infected or colonized patients. The spread of MRSA from one patient to another occurs mainly through contaminated hands of health care personnel (Solberg, 2000). The primary route of nosocomial MRSA spread between hospitals and countries is clonal dissemination of relatively few international epidemic clones.

MRSA has reached a stage where it is well adapted to the hospital environments and interestingly it is established in several hospitals in a country. Pulse field Gel electrophoresis (PFGE) is usually used for the characterization of MRSA isolates but it is not very much suitable for epidemiological studies. MLST (Multilocus sequence typing) on the other hand provides a technique for the unambiguous identification of the bacteria.

MLST has been developed and validated for *S. aureus* and provides a discriminatory method that allows related strains recovered in different countries to be readily identified (Enright et al., 2002).

2.13. MRSA IN COMMUNITY (CA-MRSA)

The term ‘Community-acquired MRSA’ (CA-MRSA) implies that the organism was acquired in the community. However, this term is often used to refer to the detection of colonization or infection in the community, rather than to actual acquisition of MRSA in the community. MRSA colonization can persist for months to years (Salgado *et al.*, 2003) and the acquisition of MRSA frequently goes unrecognized unless clinical infection develops, making it difficult to know with certainty the true site of
acquisition. The rate of MRSA colonization in the nares ranges from 8-53% (Bradley, 1999). MRSA strains of nosocomial origin may be transmitted in the community through discharged patients or health care workers. Another possibility is that MRSA strains arise de novo through acquisition of SCCmec into the genomes of previously susceptible S. aureus strains.

In 1993, it was reported in Australia in 1993 that novel MRSA strains were discovered. Again surprisingly those strains were isolated from patients who had not been exposed to any healthcare centers. (Udo et al., 1993). Publication of this information heralded the worldwide recognition of the striking evolution of genuine community acquired MRSA strains, which were transmitted in the community and differed from conventional endemic nosocomially acquired MRSA strains in several ways. First the isolated strains were susceptible to non β-lactam antibiotics (Herold et al., 1998); second, their genotypes were not the same as isolates from local hospitals (Vandenesch et al., 2003); third, they mainly harboured different methicillin-resistance cassettes (Vandenesch et al., 2003; Okuma et al., 2002 and Ito et al., 2004) and finally, community isolates were more likely to encode a putative virulence factor called Panton-Valentine leukocidin (Dufour et al., 2002).

Staphylococcus aureus is a dangerous pathogen and major public health concern. In addition to being common food poisoning agent it can cause serious skin and soft tissue infections and life threatening diseases (Micheal et al 2007)

2.13.1. Panton-valentine Leucocidin (PVL)
The genes lukS-PV and lukF-PV (pvl) encode the subunits of the Panton-Valentine leukocidin (PVL) and confer virulence (Vandenesch et al., 2003 and Berglund et al., 2005). Disease causing CA-MRSA strains possess PVL component and it is a bicomponent pore forming leukotoxin. Leukotoxin was observed to be associated with skin infections by Panton and Valentine before penicillin resistant and methicillin resistant forms of MRSA were emerged. And then after identification of PVL genes made the idea of PVL association with skin and soft tissues stronger (Gillet et al., 2002 and Lina et al., 1999) and slowly the discovery spread to other CA-MRSA isolates (Vandenesch et al., 2003) PVL positive infections are more severe than PVL negative ones (Gillet et al., 2002) Despite of large collection of toxin genes among S. aureus strains, PVL locus is the prominent one in CA-MRSA strains (Vandenesch et al., 2003 and Diep et al., 2006).

S. aureus secrete the two components of PVL, LukS PV and LukF PV, and they get assembled into a heptamer on PMN cells (Polymorphonuclear leucocytes). Higher concentration of PVL secretion causes the PMN cell to lyse and low concentrations leads to their apoptosis (Genestier et al., 2005). After lysis PMN cells release reactive oxygen species (ROS) and results in tissue necrosis. Inflammatory response can also be triggered by the release of granular contents from PMN cells. It is unlikely that PVL has a direct necrotic effect on epithelial cells.
Community acquired MRSA has been reported most often from indigenous populations (Groom et al., 2001), homeless people (Carlton et al., 2003), men who have sex with men (CDC, 2003), jailed inmates (CDC, 2003), military recruits (Zinderman et al., 2004), children in day care centres (Shahin et al., 1999), and competitive athletes (CDC, 2003). The MRSA related groups are often spread by physical contact. Antibiotic resistance patterns and genotypes of CA-MRSA usually differ from those of hospital isolates (Fey et al., 2003).

The pvl mutant can not activate the immune response unlike the parental USA300 strain and so the pvl mutant can grow in the kidney and other organs thereby compensating for any early survival advantage. If an infection is established a certain bacterial density, PVL will play only a small role in maintaining infection. (Binh et al 2008)

2.14. PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

Monitoring and limiting the intra and inter-hospital spread of MRSA strains requires the use of efficient and accurate epidemiologic typing systems that allow the discrimination between unrelated isolates and the recognition of isolates descending from a common ancestor (i.e., belonging to the same clone).

During the last four decades, multiple phenotypic and genotypic typing methods have been developed to type MRSA. The choice of a typing method depends upon the needs, skill level, resources of the laboratory and the type of question to be answered.
An optimal typing method should show high typeability, adequate stability, high technical reproducibility and high discriminatory power. Its low cost, user friendly, and ease of interpretation, and rapidity also adds up to the criteria (Struelens, 1996).

2.14.1. Phenotypic

Phenotypic characters were initially used to identify the organisms. Phenotypic typing methods characterize the strain relations indirectly through the expression of different genes. Following are some of the phenotypic methods.

**Biotyping:** Biotyping is one of the most widely utilized techniques. It is based on the differentiation of strains based on properties such as differences in biochemical reactions, morphology and environmental tolerances. Biotyping is used to determine the species to which the microbe belongs. Based upon their abilities to utilize components in different growth media and carry out certain chemical reactions, but it can also be used to differentiate different members of a same species. Today, the biotyping method is performed using automated systems and commonly used in all the labs using (Aparajita et al., 2006).

**Bacteriophage typing:** Bacteriophage (phage) typing is one of the oldest methods used for discrimination of MRSA strains; the first set of phages was established over 50 years ago (Parker, 1983). Bacteria can also be classified by the
pattern of resistance or susceptible to a particular phage, the method known as phage typing (Holmberg et al., 1984; Hopkins et al., 2004; Lina et al., 1993 and Schlichting et al., 1993). Bacteria harbors certain receptors specific to phages and this is responsible to confer resistance or susceptibility towards certain phages. The method has been applied to a number of bacteria associated with nosocomial infections, such as *S. aureus*.

**Antimicrobial susceptibility testing:** Antimicrobial susceptibility testing is a common practice in the clinical microbiology laboratory. The resultant antibiogram indicates the pattern of *in vitro* resistance or susceptibility of an organism to a panel of antimicrobial agents (Barenfanger et al., 1999 and Rudolph et al., 1998). Antimicrobial susceptibility testing is typically performed using either automated broth microdilution or disk diffusion methods. Disk diffusion and broth dilution techniques are completely standardized considering a larger platform so that these techniques are reproducible between different laboratories.

Ever since its discovery, the phenotypic detection of MRSA has been problematic. And, making the situation far more difficult, the resistant strains of MRSA have emerged. Till date, the most reliable technique used for MRSA confirmation has been the detection of *mecA* gene or its protein product (penicillin binding protein (PBP2a)) (Chambers, 1997). However, these two techniques are not universally possible by all the laboratories where the facilities are limited. And so the situation needs an alternative technique fot MRSA confirmation. According to Clinical and
Laboratory Standards Institute (CLSI), oxacillin has been used for the phenotypic tests for Penicillinase Stable Penicillins (PSPs). Recently CLSI undertook several studies to investigate the utility of cefoxitin disc diffusion test, originally proposed by Mougeot et al. (2001) and further investigated by Felton and colleagues (2002) as a potential alternative to mecA testing. A number of recent studies (Cauwelier et al., 2004; Boutiba-Ben Boubaker et al., 2004) using 30 µg cefoxitin disc diffusion method suggest greater reliability than with oxacillin.

The use of phenotypic methods for the characterization of nosocomial pathogens has been useful for our understanding of pathogens; however, these methods have drawbacks that limit their utility for highly discriminatory typing of microorganisms. Despite these limitations, phenotypic characterization continues to play a vital role in the overall management of infectious diseases. For example, routine antimicrobial susceptibility testing by the clinical microbiology laboratory may uncover a unique pattern of antimicrobial resistance, which frequently serves as early warning of potential disease problems among patients.

2.14.2. Genotypic

The shortcomings of phenotype-based typing methods have led to the development of typing methods based on the microbial genotype or DNA sequence. Genotypic typing is based on the analysis of a chromosome or extra-chromosomal DNA, allowing direct comparison of genotypes between strains. In recent years,
molecular or genotypic techniques have received increased attention as means of analyzing epidemiologic interrelationships.

5

PFGE: Pulsed Field Gel Electrophoresis (PFGE) introduced in 1984 (Schwartz and Cantor, 1984) is regarded as “gold standard” method for distinguishing MRSA strains. First, the immobilised bacterial DNA is digested with endonucleases in agarose plugs. Electrophoresis is carried out with changing electric field in regular intervals, thus separating DNA fragments of different size.(Olive and Bean, 1999) As proposed by Tenover et al., the interpretation protocol was based on results obtained from groups of S. aureus isolates from nosocomial infections. This proved the technique to be very useful making the technique applicable to even epidemiological studies as well.(Hilton et al., 2002; Montesinos et al., 2002; Wei and Chiou, 2002).

Plasmid Analysis: The first molecular method used for bacterial typing was plasmid typing (Archer et al., 1984; Eisgruber et al., 1995; Liu et al., 1996; Meyers et al., 1976). For bacterial typing, the plasmid DNA is isolated and the numbers and sizes of plasmids are compared by electrophoresis. Plasmid analysis has been applied in clinical situations to determine the evolution and spread of antibiotic resistance among isolates with different PFGE profiles or among different species of organisms within hospitals (Donabedian et al., 2003 and Feil et al., 2003). Plasmid restriction is commonly used for the analysis of staphylococci, whose plasmids are typically less than
50kb in size. The discriminatory power of this technique is increased by the inclusion of restriction enzymes.

**SLST:** Sequence data for specific loci (genes for virulence, pathogenicity, drug resistance, etc.) from different strains of the same species have revealed variability in a specific gene, such as single-nucleotide polymorphisms and areas with repetitive sequence that demonstrate potential for epidemiologic application.

At present, a gene (spa) A staphylococcal protein A gene (spa) is used for the single locus sequence typing (SLST). This protein is polymorphic due to 24bp repeat sequences that vary in sequence and number of repeats in it (Shopsin et al., 1999 and Koreen et al., 2004). Although it is applicable to *S. aureus*, spa typing appear to be very robust, with benefits in throughput, ease of use, and interpretation that tend to balance a lower level of epidemiological discrimination (Zanelli et al., 2004).

**MLST:** In 2000, Enright et al. applied Multilocus Sequence Typing (MLST) for MRSA characterization. MLST is the genomic variant of MLEE that uses sequencing variable regions of ‘house-keeping’ genes. Each new sequence is a new allele and the combination of seven alleles which forms the sequence type. The results of MLST have been used to build a database that allows comparisons among *S. aureus* isolates from all over the world. MLST excels in identifying broad population-based interrelationships. MLST demonstrates the potential of sequence-based typing to generate consistent,
reproducible isolate profiles that are highly amenable to standardization and database cataloging (Feil et al., 2004).

**Typing Methods Using PCR:** One of the first molecular techniques applied to study *S. aureus* epidemiology was the determination of the electrophoretic migration of plasmids in agarose gels. PCR is a biochemical *in vitro* reaction that permits the synthesis of large quantities of a targeted nucleic acid sequence (Mullis, 1990). A growing number of organisms have been studied using this approach, (Versalovic, 1998; Versalovic *et al.*, 1995; Versalovic *et al.*, 1991 and Welsh and McClelland, 1990). PCR technology allows the evaluation of the structural organization of the resistance determinants, such as *mecA* gene, in isolates of different origins (Lim *et al.*, 2002). In addition, some sequences can be used as targets for PCR-typing, such as the Tn916-Shine-Dalgarno (Netto dos Santos *et al.*, 2001). A different approach is the repetitive extragenic palindromic-PCR (Rep-PCR), using primers that target repetitive extragenic palindromic DNA regions. This method has been used for typing MRSA (Del Vecchio *et al.*, 1995) as well as MSSA (Netto dos Santos *et al.*, 2007) isolates.

**Multiplex PCR:** Our laboratory is involved in studying the epidemiology of *S. aureus* using different parameters from different geographical regions in south India, like Gulbarga, Raichur Hyderabad, guntur Rayalseema etc. For this present study we have selected Bangalore as another geographical location in south India. Bangalore is one of the cosmopolitan cities in south India with varied environment having large number of hospitals and health care centers. For the present study isolates have been collected from K.C General Hospital, Wockhard Hospital, Fortis Hospital and
St John’s medical college and Hospital, Institute of Preventive Medicine and Public Health Laboratories in Bangalore which receives clinical samples from both public and private hospitals in the city of Bangalore. Many of these MRSA strains are multi-drug resistant and they are characterized only phenotypically at present and molecular genotyping is not done.

There have been very few reports from India where *S. aureus* strains have been characterized by SCC*mec* and PVL analysis. Therefore, the present investigation is undertaken with the following objectives:

1. To isolate *S. aureus* from various clinical samples, healthy individuals and hospital personnel from hospitals and community in Bangalore.
2. To determine antimicrobial drug susceptibility of all *S. aureus* isolates.
3. To identify the frequency of MRSA among the study population.
4. To study the distribution of SCC*mec* types and PVL gene among MRSA isolates to correlate these with phenotypic antibiotic susceptibility pattern of the study population.

To distinguish *S. aureus* isolates from hospitals and community by phenotypic and molecular methods.

Chamberlain *et al.* (1988) developed the multiplex PCR in 1988 for staphylococci, this technique has been used to specifically detect MRSA (Martineau *et al.*, 2000), to distinguish *S. aureus* from other coagulase negative staphylococci. S (Mason *et al.*, 2001) and to detect several resistance determinants (Martineau *et al.*, 2000) and
staphylococcal toxin genes simultaneously (Sharma et al., 2000). PCR is considered the ‘gold standard’ (Prasad et al., 2000). It is rapid with high degree of sensitivity and specificity, but is expensive. A combination of primers for genes responsible for coagulase activity (femB) and methicillin resistance (mecA) would identify all the isolates and can be used to perform a single step multiplex PCR (Kobayashi et al., 1994; Vannuffel et al., 1995).

A new approach to study MRSA is the Staphylococcal Cassette Chromosome mec (SCCmec) typing using multiplex PCR. Multiplex PCR is the modified form in which multiple sets of PCR primers are used in a single reaction tube. This increases the PCR efficiency and reduces reagent costs. (Focucault et al., 2005 and Francois et al., 2004). The genetic element SCCmec, described by Katayama et al. (2000), is responsible for the mobilization of the mecA gene and harbours ccrA, ccrB or ccrC genes which encode chromosome recombinases, and the mec complex. In 2002, Enright et al. analyzed an international collection of hospital and community-acquired S. aureus isolates by MLST and SCCmec typing. The analysis revealed new information about clonal groups of S. aureus.

2.15. PRESENT STATUS OF S. AUREUS IN HOSPITALS AND COMMUNITY

MRSA nosocomial infections were mainly detected in large tertiary hospitals and in intensive care units, where colonized and infected patients as well as colonized health-care workers were a significant source of cross-infection. Currently, MRSA is one of the most common pathogens in hospitals of all sizes worldwide.
2.15.1. International status

Methicillin resistant *S. aureus* is an important pathogen causing pyogenic, disseminated and toxin mediated infections (Enright *et al.*, 2002; Oliviera *et al.*, 2002). In a study conducted in Korea, to investigate the genotypic characterization of MRSA, 74 MRSA strains isolated from 12 Asian countries were analyzed by MLST and SCCmec typing. In some Asian countries such as Taiwan, china and korea, the resistance of methicillin resistance is more than 70%. (de Souset *et al.*, 2003; Kim *et al.*, 2003; Huang *et al.*, 2004).

For evaluating the epidemiology of MRSA and for the strain characterization, PVL analysis and SCCmec typing act as valuable tools. The SCCmec typing provides strong evidence for the independent deviation of HA-MRSA and CA-MRSA clones. Shannon *et al.* (2006) reported the distribution of SCCmec types in HA-MRSA and CA-MRSA. The SCCmec types I, II and III are predominantly found in HA-MRSA strains whereas the SCCmec type IV is mainly associated with CA-MRSA throughout the world.

Donnio *et al.* (2004) investigated the 11 year old antibiotic resistant profiles of *S.aureus* isolates and the relationship between changes in antibiotic resistance and distribution of SCCmec types among MRSA in hospitals of Rennes and France. A correlation of $\geq 3$ was observed between isolates and susceptibility profiles.
O’Brien et al. (2004) performed MLST, spa typing, and antibiograms to compare overall relatedness of isolates from Australia. The study emphasizes the diversity of CMRSA found in Australia and the importance of typing in tracing the origin of isolates and in designing antibiotic policies for their control in the community.

Abdullah et al. (2006) determined the SCCmec types and occurrence of the PVL gene and correlated them with the phenotypic antibiotic susceptibility patterns for MRSA strain isolated from children and adults at Vanderbilt University Medical Center (VUMC) during a 12 months study period. The study focused on the differences between children and adults due to the perception that children were having an increased incidence of serious staphylococcal infections.

In a study, conducted in a hospital in Taiwan, 190 isolates were typed to determine the predominant SCCmec element in MRSA. All isolates were tested by SCCmec element typing and Multi-Locus Sequence Typing (MLST) and were analyzed for PVL gene and antimicrobial susceptibility to a panel of selected antibiotics (Jann-Tay et al., 2007).

Paul and Charles (2007) in their study assessed the antimicrobial susceptibility patterns and prevalence of methicillin resistance among S. aureus isolates from hospital and community sources in southern Jamaica.
Gurudabassi et al., collected nasal swabs from 1600m pigs and MRSA characterization was carried out using protein A (spa), multilocus sequence typing and toxin genes detection. Also a case study was carried out comparing risk factors associated with MRSA in regional pigs and patients. On about 70% of the farms MRSA carried by pigs were identified (spa types t011, t034, t108, t1451 and t2510, all associated with MLST sequence type ST398). For the carriage of these spa types, contact to pigs and cattle were considered as independent risk factors. Livestock is the main source of the MRSA into hospitals (Rock et al.2009).

The prevalence of MRSA in hospitals continues to increase worldwide. International comparisons indicate that there is considerable variation in prevalence of MRSA among hospitals and countries within a region.

2.15.2. National status

India. The widespread emergence of MRSA, especially in various types of nosocomial infection, is a serious clinical problem and there is a drastic increase in MRSA prevalence rate in Indian hospitals. Most of the studies have reported the prevalence of MRSA to the extent of 20% to 32% (Mehta et al., 1997).

More recently, some of the studies, have reported the incidence of methicillin resistance to phenomenal proportions in Indian hospitals, with some cities reporting the incidence upto 70% and a high level of antibiotic resistance among the isolates of MRSA (Verma et al., 2000; Vidhani et al., 2001; Anupurba et al., 2003).

Risk factors for hospital acquired MRSA include prolonged hospitalization, antimicrobial therapy, being in an ICU or burns ward and being in close proximity to a patient colonized or infected with MRSA. The Staphylococcus aureus strains isolated
from hospitals in Bangalore possess 40-50% of MRSA strains (Krishnan et al., 2002). To determine the prevalence of MRSA colonization in health-care workers, two third of the hospital personnel working in burns ward were positive for the MRSA. The problem is confounded in burns unit as patients are severely immuno-compromised and receive numerous antibiotics (Preetha et al., 2000).

In recent years MRSA has become a particularly significant problem in Indian hospitals (John, 1996). In a study conducted in a tertiary care hospital in India, MRSA carriage rate ranged between 28.4% in out patients to 33.5% in in-patients (Mathur et al., 1994).

Rajaduraipandi et al. (2006) reported the prevalence of MRSA from different clinical and carrier screening samples and there in vitro susceptibility pattern to various antimicrobial agents.

It has been reported that the hospital workers, infected patients are the major sources of staphylococci. These sources pose risk in transmitting infection, from healthcare workers to patients and patient to patient which is the major mechanism for transmitting the disease. A study was conducted on molecular epidemiology of clinical and carrier isolates of MRSA in the hospital settings of north India. The highest percentage (35.5%) of MRSA in pus specimens and the highest resistance was found to penicillin-G followed by ampicillin. PFGE typing of clinical and carrier isolates was performed (Dar et al., 2006).

Initially *Staphylococcus aureus* was confined to hospitals but recently it has begun to appear in the community as well and has shown resistance to methicillin. This changing epidemiology has prompted many researchers to study the nasal carriage of MRSA amongst healthy individuals in community. Various hospital based studies have described the incidence of MRSA and carriage of this organism in healthcare workers. Saxena et al. (2003) assessed the carriage rate of MRSA in healthy population with no recent exposure to hospital or health-care workers and reported higher susceptibility rate among the strains obtained from carrier screening samples.
The epidemiology of clinical and carrier isolates of MRSA in the hospital settings of north India. The highest percentage (35.5%) of MRSA in pus and nasala specimens and the highest resistance was found to penicillin-G followed by ampicillin. PFGE typing of clinical and carrier isolates was performed the laboratory by (Deurenber., et al 2007).

In the past few years molecular methods have made a way for sophisticated techniques and to track the source and transmission route of bacterial pathogen by (Juhasz-Kaszanyitzky E., et al 2007). The genotyping of Indian staphylococcus is not yet completed but a single report by Hanssen et al who distinguished two Indian isolated by PFGE typing and published that these isolated belong to ccr type 3.

Among 82 strains from two hospitals in Bangalore that were genotyped (Arakere et al., 2005) 75 isolates were type III or IIIA The diversity was found in the lot of pus samples obtained from St. Johns hospital by PFGE analysis and it was observed that the types III and IIIA were equally distributed. The staphylococcus strains from St. Johns hospital and Manipal hospital and found that they comprise of 43% with pus and 38% with miscellaneous sources.. However, PFGE, MLST and spa typing data indicate that most of the strains obtained from two hospitals are genetically related and are clonally similar to the Hungarian and the Brazilian epidemic strains (Nadig et al., 2006).

penicillin resistance controls the mecA transcription. And this mecA gene is embedded in a polymorphic chromosomal cassette known as SCCmec element. Where in order to assess the eventual correlation between bla allotypes and genetic lineages,(Milheiriço 2011)

A thorough understanding of the molecular epidemiology and evolution of MRSA is required to help detect, track, control and prevent human diseases due to this organism (Ubukata et al., 1985). The discriminate power of most of phenotypic methods is restricted and ambiguous (Projan and Novick, 1997 and Gordon, 1998). Molecular typing methods have in the last few years paved the way, for sophisticated technique to track the source and transmission route of bacterial pathogens. The complete characterization of MRSA needs genetic background, and the SCCmec complex elements (Ubukata et al., 1985). Antimicrobial resistance profile of each strain of MRSA, therefore can be evaluated by detecting the resistance genes identified on SCCmec.
The overall effectiveness of empiric therapy can be improved and infections can be treated in their early stages before the pathogen overwhelms the patient (Matsuhashi et al., 1986).

It has been reported that PVL determinant is common in *Staphylococcus aureus* isolated from community but is rare among the isolates of hospitals. And therefore PVL can be used as a useful marker for the identification of community acquired MRSA worldwide (Ubukata et al., 1985).

This study was conducted for a better understanding on the epidemiology of *Staphylococcus aureus* and to enhance therapy and management of patients in Nigeria. Analysis were performed on a few *Staphylococcus aureus* isolates from clinical samples of healthy medical personnel in two states named Ondo and Ekiti from south Nigeria (Strommenger Bet al 2008). PFGE, PCR-RFLPs, antibiotic susceptibility pattern was carried out for the 54 isolates out of which 50 were confirmed to be staphylococcus aureus. And all the isolates were susceptibility to fusidic acid, clindamycin, but resistant to penicillin, tetracycline, erythromycin, gentamicin and ciprofloxacin (Esan et al 2009).

*Staphylococcus aureus* cause different clinical syndromes, they differ in antimicrobial susceptibility Patterns, they spread rapidly among healthy people in the community, and they Frequently cause infections in health care environments as well. This review details what is known about the epidemiology of CA-MRSA strains and the clinical spectrum of Infectious syndromes associated with them that ranges from a commensal state to Severe, overwhelming infection. It also addresses the therapy of these infections and Strategies for their prevention (David et al 2010)

After the cross-sectional study of adults and children with *Staphylococcus aureus* skin infections and their household contacts in Los Angeles and Chicago. Subjects were surveyed for *Staphylococcus aureus* colonization of the nares, oropharynx, and inguinal region and risk factors for *Staphylococcus aureus* disease. All isolates underwent genetic typing (miller 2012). 7

ClaI digested staphylococcus DNA was used for hybridization techniques with mecA ans Tn554 specific probes for the characterization and determination of macrorestriction
patterns. Smal digested chromosomal DNA was also subjected to PFGE and found that 76% of the isolates harbor Tn554 pattern A and PFGE pattern A (Desousa, et al 2003). According to zaffar, the study of staphylococcal cassette chromosome mec (SCCmec) typing and multilocus sequence typing (MLST). PFGE identified nine pulsotypes, which were predominant among hospital-onset MRSA (HO-MRSA) and community-onset MRSA (CO-MRSA) isolates, respectively. Variants of SCCmec type III were prevalent in HO MRSA and in CO MRSA isolates, SCCmec type IV were predominant (Zaffar et al 2010).

Particularly those displaying multidrug resistance, for the treatment of staphylococcus species, bacteriophage endolysins have become an effective alternative for the antibiotics. The only hurdle in continuing this technique is only due to their low solubility their purification and production in large scale seems very difficult. Many substitutions like the use of staphylococcal endolysin in place of cell wall binding domains were unsuccessful. But after certain standardisations, the chimeras showed high solubility and also showed broad activity even against MRSA strains and other gram positive bacteria (Cantate et al 2012).

There have been very few reports from India where Staphylococcus aureus strains have been characterized by SCCmec and PVL analysis. Therefore, the present investigation is Undertaken with the following objectives:

Our laboratory is involved in studying the epidemiology of S. aureus using different parameters from different geographical regions in south India, like Gulbarga, Raichur Hyderabad, guntur Rayalseema etc. For this present study we have selected Bangalore as another geographical location in south India. Bangalore is one of the cosmopolitan cities in south India with varied environment having large number of hospitals and health care centers. For the present study isolates have been collected from K.C General Hospital, Wockhard Hospital, Fortis Hospital and St John's
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9. To distinguish S. aureus isolates from hospitals and community by phenotypic and molecular methods.