Review of Literature
2. REVIEW OF LITERATURE

2.1 History of MRSA

The first semi synthetic penicillin namely methicillin was introduced in 1959 to overcome the problems that arose from the increasing prevalence of penicillinase producing \textit{S. aureus} resistant to penicillin (Livermore, 2000). During the 1980s methicillin resistant \textit{S. aureus} (MRSA) started to constitute a widespread human health concern (Chambers, 1997).

Methicillin-resistant \textit{S. aureus} (MRSA) was first reported by Jevons in 1961. Throughout the 1960s and 1970s, these organisms were responsible for sporadic, although sometimes series outbreaks of hospital infections (Casewell, 1986; Jepsen, 1986). In the United States, few outbreaks of hospital infections due to MRSA were reported before 1980; however, since then, MRSA has caused increasing problems in hospitals in the US (Thompson et al., 1982) and worldwide (Townsend et al., 1987).

2.2 Methicillin Resistant \textit{Staphylococcus aureus} in wound

\textit{Staphylococcus aureus} is one of the most important pathogens that cause suppuration, abscess formation, a variety of pyogenic infection, and even fatal septicemia in human beings. \textit{Staphylococcus aureus}, which can induce bacteremia (associated with 80% mortality in the preantibiotic era), proved to be susceptible to the earliest antimicrobial substance; however, as antibiotic use increased, staphylococcal resistance rapidly developed (Bramley et al., 1989; You et al., 1999). The first is a habitual host of the skin and along with \textit{S. epidermidis} is the most frequent agent of skin infections. \textit{S. aureus} mainly causes wound infections and boils. On the other hand, \textit{Pseudomonas
*Pseudomonas aeruginosa* is gram negative bacteria that can infect and complicate burns and traumatic wounds (Iovine and Selva, 1985).

The less of the natural cutaneous barrier to infection and the presence of coagulated protein and other microbial nutrients in the burn wound, combined with a vascularity of the wound tissue, head to microbial colonization (Mayhall, 2003). Colonization is invariably followed by invasion of microorganisms, giving rise to burn wound infection. The burn wound and blood stream infection rates were 14 and 54 infections per 100 patients, respectively (Cerda *et al.*, 2001).

Patients with burns acquire MRSA more frequently than other acutely ill surgical patients (Phillips *et al.*, 1992). *S. aureus*, both sensitive and resistant to methicillin, has been recognized as the predominant PPM in burn patients (Taylor *et al.*, 1992; Oncul *et al.*, 2002). Burn patients can import or acquire MRSA that contaminates the environment and the hands of health care workers. Patient to patient transmission via hands is the most common mode for spread and subsequent outbreaks leading to endemicity. MRSA may cause significant and even lethal infections, with an associated mortality of 20 to 40% among those clinical infections (Collopy *et al.*, 1984; Pavillard *et al.*, 1982).

Hospital-acquired infections with MRSA pose a major threat to patients admitted to many UK hospitals. The proportion of colonized patients who become infected varies between 5-60% depending on the study population and strain of MRSA. Predisposing factors to superficial colonization include length of stay and previous use of antimicrobials. The risk of colonization progressing to infection is increased in the presence of any skin breach e.g. surgical wounds and devices penetrating the skin such as prostheses and catheters. *Staphylococcus aureus* is carried in the nostrils or on the skin of about 30% of the population. This percentage increases to about 50% in healthcare workers or hospital in patients. *S. aureus* can cause serious infections including skin and soft tissue infections, wound infections, bacteraemia (blood stream infections), pneumonia and endocarditis. The main transmission is through direct contact via the transiently
contaminated hands of healthcare workers, although airborne and environmental transmission also occurs (Cooper et al., 2003).

2.3 Community acquired MRSA

Staphylococci are the most common etiologic agents of nosocomial bloodstream infections (Marshall et al., 1998). Methicillin-resistant *Staphylococcus aureus* has been recognized as an important pathogen for both hospitalized patients and possibly for community-acquired infections (Boyce, 1998; Herold et al., 1998; Mulligan et al., 1993).

There have been an increasing number of reports of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA). Boyce (1998) has reviewed the evidence for community-acquired MRSA and pointed out the difficulties in demonstrating that the organism was not acquired as the result of a visit to a health care facility and also pointed out that many community MRSA strains are not multiple resistant and that some of these may be borderline methicillin-resistant strains, which do not contain the *mecA* gene.

2.4 Vancomycin Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA), resistance of which was due to β-lactamase production, was isolated in the early 1960s. Since resistance due to penicillin-binding protein 2', so-called intrinsic resistance (Utsui and Yokota, 1985) was acquired, intrinsic MRSA multi-resistant to various antibiotics has been emerging pathogens in hospitals (Mulligan et al., 1993). Certain antibiotics are usually chosen for the treatment of MRSA infections. However, their use not only suffers from unexpected side-effects but also reduces susceptibility to them by target site alteration enzyme modification and permeability change to select resistant strains (Brumfitt and Hamilton-Miller, 1989). Although vancomycin is recognized as the most effective agent for MRSA (Craven et al., 1983).
*Staphylococcus aureus* is one of the most common causes of hospital and community acquired infections. Since the recognition of vancomycin resistant enterococci in 1988, the emergence of vancomycin resistant *S. aureus* (VRSA) (Minimum inhibitory concentration ≥32μg/ml) has been anticipated. The transfer of the genetic element containing the VanA vancomycin resistance gene from *Enterococcus faecalis* to *S. aureus* was demonstrated in the laboratory in 1992; the first clinical infection with VRSA was reported in July 2002 (CDCP, 2002).

In 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan (Hiramatsu *et al.*, 1997). Since this report, three cases of *S. aureus* with reduced susceptibility have been reported in the United States of America and one in France. A further clinical specimen has been isolated in Slovakia, although confirmation that it truly has reduced susceptibility to vancomycin has not yet been established (Tabaqchali, 1997).

The vancomycin minimum inhibitory concentration (MIC) result reported for this isolate was in the intermediate range (Vancomycin MIC=8 μg/ml) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards (NCCLS). As of June 2002, eight patients with clinical infections caused by vancomycin-intermediate *S. aureus* (VISA) have been confirmed in the United States. This report, describes the first documented case of infection caused by vancomycin-resistant *S. aureus* (VRSA) (Vancomycin MIC≥32 μg/ml) in a patient in the United States. The emergence of VRSA underscores the need for programs to prevent the spread of antimicrobial-resistant microorganisms and control the use of anti-microbial drugs in health-care settings (Smith *et al.*, 1999; Fridkin, 2001).

Vancomycin was identified in 1956 as result of intensive efforts to screen natural specimens for new antibiotics with activity against staphylococci in which resistance to the available agents had already been recognized as an emerging treat vancomycin was approved by the US FDA in 1958 (Finch and Eliopoulos, 2005).
The vanA vancomycin resistance gene could be transferred from vancomycin resistant Enterococcus faecalis to S. aureus. Recently described isolation of a vancomycin and methicillin resistant S. aureus strain (VRSA), Mu 50 (MIC of Vancomycin=8mg/l) from the Japanese patient with a surgical wound infection who failed to respond to vancomycin therapy. The isolation of Mu50 was preceded by the isolation of another methicillin resistant S. aureus (MRSA) strain, Mu3, from another Japanese patient with post surgical pneumonia who also failed to respond to vancomycin therapy (Hiramatsu et al., 1997).

2.5 DISTRIBUTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

2.5.1 MRSA in India

Consecutive, non repeat clinical isolates of MRSA from patients admitted to University College of Medical Sciences and Guru Tegh Bahadur hospital, Delhi, over a period of one year from January 2002 to January 2003, Methicillin resistance was detected based on National Committee for Clinical Laboratory Standards (NCCLS) recommendations using 1g of oxacillin on a swab inoculated MHA plate supplemented with 2% NaCl (NCCLS, 2000).

Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen in India, up to 70% methicillin resistance has been reported from hospitals in various parts of India. Arakere et al., (2005) reported that the first time, hospitals use phenotyping for the most part, and molecular genotyping is not done. The authors were also reported that the genotyping of 82 single-patient isolates from two hospitals in Bangalore, South India.
Genotyping data from large international studies have shown that a few clones of MRSA are responsible for the spread of the disease in various parts of the world (Chung et al., 2004; Enright et al., 2000; Oliveira et al., 2002). About 40 to 50% of S. aureus strains isolated from the burn and trauma wards in hospitals in and around Bangalore, India (Krishnan et al., 2002). Many of these MRSA strains are multidrug resistant and they are characterized by phenotypically. The discriminatory power of most of the phenotypic methods is restricted and ambiguous (Ip et al., 2003; Schlichting et al., 1993). Molecular typing methods have in the last few years paved the way for sophisticated techniques to track the source and transmission route of bacterial pathogens in hospital outbreaks and have also helped in establishing epidemiological investigations comparing strains across continents (Aires de Sousa et al., 2003; Chung et al., 2004; Straden et al., 2003).

### 2.5.2 MRSA in Australia

Western Australia (WA) has been able to prevent methicillin-resistant *Staphylococcus aureus* (MRSA) strains from outside of the state from becoming established in its hospitals. A single-strain outbreak of MRSA occurred in a WA metropolitan teaching hospital following admission of an infected patient from a remote community. The strain responsible for the outbreak was unrelated to any imported strains and spread rapidly in the hospital. Screening of two remote communities in the region from which the index case came revealed that 42% of the people in one community and 24% in the other carried MRSA. Community MRSA strains were first reported in the state of Western Australia (WA) in 1993 (Udo et al., 1993). These strains were isolated from hospital patients who resided in isolated communities hundreds of kilometers from any small town and thousands of kilometers from any urban area. The strains differed from other MRSA strains previously isolated in WA (Townsend et al., 1985), based on their resistance to antimicrobial agents, plasmid content and contour-clamped homogeneous electric field (CHEF) electrophoresis patterns (Udo et al., 1993) and have been referred to as WA MRSA (Udo et al., 1994).
Marples and Reith (1996) demonstrated that a community strain of MRSA was responsible for a single-strain hospital outbreak. A single-strain outbreak in a hospital is defined as an outbreak when three or more patients are infected or colonized by isolates of MRSA which are indistinguishable by at least two typing techniques and the strain has been transmitted to at least two patients within the hospital. Epidemic strains of MRSA (EMRSA) are strains which have caused single-strain out-break in two or more hospitals.

2.5.3 MRSA in Japan

The increasing prevalence of benzylpenicillin-resistant \textit{S. aureus} was initially over come by the introduction of semi synthetic penicillins. Since that time, methicillin-resistant \textit{S. aureus} has rapidly emerged and become a major clinical problem. In 1996, 57% of \textit{S. aureus} isolates from French patients with nosocomial infections were methicillin resistant (Astagneau and the French prevalence survey study Group). Epidemiological studies on high-level MRSA, which is resistant to numerous antibiotics and antiseptics, revealed nosocomial outbreaks with clones disseminating nationally and internationally (Ayliffe, 1997). Infections caused by very-low-level MRSA were first reported in Japan, where MRSA was more prevalent than elsewhere (Hiramatsu, 1995; Okonogi et al., 1989).

2.5.4 MRSA in US and Canada

Various hospital-based studies have described the incidence of MRSA causing such (Nosocomial) infections (Thompson et al., 1982; Salaria and Singh, 2001). In Guru Tegh Bahadur (GTB) Hospital, 33\% \textit{S. aureus} infections were found to be caused by MRSA, while the nasal carriage rate of this organism in health care workers was reported to be 39\% in 1995. Until a few years ago, only nosocomially acquired isolates were found to show such resistance, but recently even community-acquired strains have shown resistance to methicillin (Herold et al., 1998; O'Brien et al., 1999). Reports of increasing community acquired MRSA from the United States, Canada and Australia are evidence of
the changing epidemiology of MRSA. Because of such changes it is important to assess the carriage rate of MRSA in the community amongst healthy individuals who have not been hospitalized nor had antibiotic therapy in the recent past.

50.9% of *S. aureus* isolates in North America were found to encode and express the *mecA* gene. When the occurrence was analyzed by country, the occurrence of MRSA in the United States (129 isolates) was nearly 64% whereas the incidence in Canada (36 isolates) was 5.5%. These data continue the upward trend seen in earlier studies (Biedenbach *et al.*, 2004; Diekema *et al.*, 2001; Doern *et al.*, 1999). Biedenbach *et al.*, (2004) reported the incidence for blood stream isolates in North America to be 39.1% with a 43.5% rate of occurrence among US isolates. The difference in incidence is most likely affected the demographics of the patient population in the various studies.

2.5.5 MRSA in Taiwan

In Taiwan, MRSA was first documented in the early 1980s (Chang *et al.*, 1998). The incidence of nosocomial MRSA infections increased remarkable in the 1990s (Chang *et al.*, 1997). In 2000, MRSA accounted for 53 to 83% of all *S. aureus* clinical isolates in 12 major hospitals (Huseh *et al.*, 2002) and they believe that this is also true in most large hospitals in Taiwan.

Geographic spread of one or several MRSA clones in a city (Roberts *et al.*, 1998; Roman *et al.*, 1997) in a country (Aucken *et al.*, 2002; De Lencastre *et al.*, 1997; Salmenlinna *et al.*, 2000; Teixeira *et al.*, 1995; Van Belkum *et al.*, 1997) even between countries (Santos Sanches *et al.*, 1995; Townsend *et al.*, 1987) and continents (Aires de Sousa *et al.*, 1998; Ayliffe 1997) has been reported and proven by molecular evidence. In Taiwan, the islandwide molecular epidemiology of MRSA isolates has not yet been studied extensively, although a study has been reported (Wang *et al.*, 2002).
2.6 SCREENING OF METHICILLIN RESISTANT \textit{STAPHYLOCOCCUS AUREUS}

Screening for MRSA is important for therapeutic and epidemiological reasons. Methods used to detect MRSA in clinical samples ideally should have high sensitivity and specificity and should report the results within a short time. To identify \textit{S. aureus} from contaminated samples more easily and reliably, selective media have been developed. Ideally, selective media achieve isolation of \textit{S. aureus} and detection of methicillin resistance in one step (Simor \textit{et al.}, 2001).

2.6.1 Sensitivity of CHROM Agar

The use of screening cultures to identify MRSA-colonized patients so that infection control measures can be implemented and prevent transmission to other patients, is well established (Salgado and Farr, 2006). The guidelines of the society for Healthcare Epidemiology of America emphasize the importance of identifying reservoirs of nosocomial transmission by use of active screening methods (Muto \textit{et al.}, 2003). However, traditional methods used to screen for MRSA rely on labor intensive and time consuming culture techniques which do not exclude MRSA for 48 hrs and may require a further 1-2 days to confirm positives. The use of chromogenic media may reduce the time significantly, but there are few published studies on their efficacy (Brown \textit{et al.}, 2005). During this time period, infection control measures, such as isolation, or cohorting of patients and prophylactic decontamination may be applied unnecessarily or if not applied, unidentified MRSA-positive individuals may remain a hidden reservoir for cross infection. A rapid negative result should allow more effective use of hospital isolation resources, whilst a rapid positive result should help reduce the spread of infection and MRSA infection rates.

Merlino \textit{et al.}, (2000) found that the adapted media to be effective for the growth of multidrug - resistant Methicillin Resistant \textit{S. aureus} strains but less effective for the growth of community - acquired MRSA strains. Kluytmans \textit{et al.}, (2002) examined that
the utility of CHROM agar *Staphylococcus aureus* supplemented with 4 mg of oxacillin/liter.

Perry *et al.*, (2004) described that the development and evaluation of MRSA ID, a new chromogenic agar medium for the specific isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) and also used *S. aureus* ID (Bio Merieux, La Balme Les Grottes, France) and supplemented it with various antimicrobials, including cefoxitin, ciprofloxacin, oxacillin and methicillin.

A new chromogenic plate medium, CHROM agar *S. aureus* (CHROM agar, Paris, France), for the identification of *Staphylococcus aureus* on the basis of colony pigmentation. The abilities of CHROM agar *Staphylococcus aureus*, thermostable nuclease (DNase) and mannitol salt agar (MSA) to identify *S. aureus* isolates (n=114) and discriminate between *S. aureus* and coagulase-negative staphylococci (CoNS; n=22) were compared (Merlino *et al.*, 2000).

### 2.6.2 Sensitivity of ORSAB

Becker *et al.*, (2002) reported that the sensitivity of ORSAB was as high as that reported by Simor *et al.*, (2001), but positive predictive values were much lower (48.4%) in the study of Becker *et al.*, (2002). This discrepancy may be explained by the fact that Becker *et al.*, included all submitted clinical specimens, rather than restricting the experiment to patients at high risk for MRSA colonization. This inclusion will likely result in a lower prevalence of MRSA and consequently, a lower positive predictive value.

Blanc *et al.*, (2003) evaluated the sensitivity of ORSAB medium for the recovery of MRSA from patients' specimens by using ORSAB alone as a primary culture medium and as a subculture of a selective enrichment broth (Mueller-Hinton broth supplemented with NaCl and oxacillin). A low sensitivity (74%) was obtained when ORSAB medium was used alone as a primary culture, whereas the sensitivity was 88% when a single
selective enrichment broth was used. The specificity was only 47% in both cases. The author concluded that an enrichment broth is still needed to ensure a good sensitivity for the recovery of MRSA.

2.6.3 Other Sensitivity medium

Gurran et al., (2002) reported that to be superior to the use of Baird-Parker medium plus ciprofloxacin and mannitol salt agar plus 4 mg of oxacillin/liter for the isolation of MRSA. A wide range of methods has evolved for the detection of MRSA in the clinical laboratory (Apfalter et al., 2002; Gurran et al., 2002; Merlino et al., 2003), cultural methods using selective media are predominantly used and usually employ oxacillin or methicillin to differentiate MRSA from methicillin-sensitive S. aureus (MSSA).

Considerable effort has been expended by numerous investigators (Merlino et al., 1996; National Committee for Clinical Laboratory Standards, 1993; O’Brien et al., 1999) in the development of reliable media for differentiation of S. aureus from coagulase-negative staphylococci (CoNS). S. aureus has most frequently been associated with the coagulation of plasma, the fermentation of mannitol (mannitol salt agar [MSA], the production of thermostable nuclease (DNase), egg yolk lipase hydrolysis (lipovitellin-salt-mannitol agar [LSM]) and the production of natural pigment (Merlino et al., 1996).

Enk and Thompson, (1992) recommended using an oxacillin containing selective and differential medium for MRSA screening and as a primary plating medium if it is important to detect low levels of MRSA. They have indicated that approximately 30% of MRSA containing clinical specimens may be missed if only nonselective media are used for primary specimen plating.
2.7 MOLECULAR CONFIRMATION OF METHICILLIN RESISTANT

STAPHYLOCOCCUS AUREUS

2.7.1 Polymerase Chain Reaction (PCR)

Molecular typing techniques have been used with increasing frequency in studies of the epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) and also for a better understanding of the evolutionary relationships among MRSA clones (Crisostomo et al., 2001; Enright et al., 2000; Olivera et al., 2001). One of the conclusions emerging from these studies was that a complete characterization of MRSA lineages requires not only identification of the genetic background of the bacteria but also identification of the structural types of the large and heterologous mec element, which carries methicillin resistance determinant mecA (Hiramatsu et al., 2001; Oliveira et al., 2001; Oliveira et al., 2002).

Multiple-antibiotic-resistant S. aureus strains constitute a major health care problem; therefore, a availability of sensitive and specific methods for the accurate detection of antibiotic resistance in these bacteria has become an important tool in clinical diagnosis. Since phenotypic typing methods are not discriminating enough and are highly dependent on growth conditions, it is essential to use molecular techniques to stop the spread of multiple-antibiotic-resistant S. aureus. These techniques allow a rapid, accurate identification of staphylococci and their resistance type. Thus, fast, sensitive, and specific molecular methods will be an essential diagnostic tool for microbiology laboratories. The use of PCR for the sensitive and specific detection of microorganisms and antibiotic resistance gene is increasing in clinical microbiology laboratories (Nunes et al., 1999; Tokue et al., 1992; Vannuffel et al., 1995).
2.7.2 Detection of *mecA* gene

A polymerase chain reaction (PCR) based test was developed for the detection of *mecA* in staphylococci. To facilitate this process, a rapid cell lyses procedure was established for the release of DNA from Staphylococcal strains. Primers based on the DNA sequence of the *mecA* gene from *Staphylococcus aureus* were used in PCR, to screen for the presence of gene (Unal *et al.*, 1992).

Multiple resistant *S. aureus* has acquired successively a number of resistance determinants by acquisition of plasmids and transposons. Located on an additional 30-40 kb DNA segment of the *mecA* gene, which encodes for methicillin resistance is always chromosomal. It codes for the additional penicillin binding protein (PBP), PBP2a which has low affinity for all β lactam antibiotics (Archer and Niemeyer, 1994). As is evident from many surveillance studies, and there is a worldwide increase in the prevalence of MRSA among *S. aureus* strains. There are however considerable differences between individual countries (Witte, 1999).

2.7.3 Borderline Error

The *mecA* gene is unique to MRSA. The DNA sequences of the *mecA* genes found in *S. epidermidis* and *S. aureus* are >99% identical (Wu *et al.*, 1992). Therefore, the *mecA* gene represents a useful molecular handle for rapid identification of MRSA and MRSE by PCR. Detection of *mecA* is not subject to growth conditions and may be applicable to a ramen broad range of staphylococci. Another gene, *femA*, has been characterized as essential for the expression of methicillin resistance in *S. aureus* (Berger-Bachi *et al.*, 1989).

Very-low-level methicillin-resistant *Staphylococcus aureus* (MRSA) or class 1 MRSA, is often misdiagnosed as methicillin-susceptible *S. aureus* (MSSA). Felton *et al.*, (2002) evaluated the performances of three methods for detection of low-level methicillin
resistance: the disk diffusion method using the cephamycin, cefoxitin and moxalactam, the vitek 2 system (bio Merieux), and the MRSA-screen test (Denka). Detection of the mecA gene by PCR was considered to be the "gold standard". The authors were also determined the sensitivity of the oxacillin disk diffusion method with 5-and 1-μg disks and that of the oxascreen agar assay with 6 mg of oxacillin liter⁻¹ for detection of MRSA.

2.7.4 Golden Standard Method

Currently, a number of technologies are available for the detection and confirmation of MRSA (Arbique et al., 2001; Louie et al., 2000; Nicola et al., 2000). Several commercial kits have been developed for the rapid confirmation of MRSA following growth on oxacillin (6μg/ml)-supplemented agar. However, multiplex PCR still remains the "gold standard" for the confirmation of MRSA through direct detection of the mecA gene in clinical isolates and distinguishing between MRSA and borderline oxacillin-resistant strains (Nicola et al., 2000).

Roth et al., (2001) described a multiplex PCR assay for the detection of clinically relevant antibiotic resistance genes harbored by some Staphylococcus aureus isolates and for the simultaneous identification of such isolates at the species level. Conditions were optimized for the simultaneous detection of the 310, 456, and 651-bp regions of the mecA (encoding high-level methicillin resistance). This assay offers a rapid, simple, feasible, specific, sensitive and accurate identification of mupirocin-resistant MRSA clinical isolates and could be systematically applied as a diagnostic test in clinical microbiology laboratories, facilitating the design and use of antibiotic therapy.

Multiple-antibiotic resistant S. aureus strains constitute a major health care problem, since they are the etiologic agent of several nosocomial and community-acquired pathological infections. For that reason, accurate and fast detection of resistant isolates constitutes a critical goal of clinical microbiology and therefore, PCR assays have become an essential tool in laboratory programs. Although previous reports have evidenced the
utility of PCR for the accurate detection of the mecA gene (Schmitz et al., 1997; Tokue et al., 1992) and the possibility of simultaneous identification of S. aureus and detection of mecA (Geha et al., 1994; Jonas et al., 1999; Salisbury et al., 1996; Tenover et al., 1999; Towner et al., 1998; Vannuffel et al., 1998).

The mecA gene, which encodes penicillin-binding protein PBP 2a, correlates with methicillin resistance in coagulase-negative staphylococci (Chambers, 1993; Kloos and Bannermann, 1994; Murakami et al., 1991). In an effort to improve the detection of resistance, methods to easily and rapidly detect the mecA gene have been recommended for routine laboratory use (Archer and Pennell, 1990; Brakstad et al., 1993; Geha et al., 1994). Although these methods are feasible for some laboratories, most clinical laboratories do not have the resources to efficiently develop and perform PCR or DNA probe techniques routinely.

Detection of the mecA gene in the strains of MRSA was performed by PCR application. Total genomic DNA was obtained from Staphylococcus aureus by the phenol chloroform extraction method as described earlier in the previous report (Tsen and Chen, 1992).

2.8 BIOACTIVE COMPOUNDS

In 1967, 58% of antibiotics were produced by actinomycetes, 18% by fungi, 12% by higher plants, 9% by bacteria and the remaining 3% by algae, lichens and animals (Edwards, 1980). Studies by noristan on more than 300 plants from Southern Africa showed that 31% had a high activity, 48% a medium activity and 21% no activity when tested against a range of pharmacological parameters (Fourie et al., 1992).

Leaves were reported to be the most frequently used part of the plants for the treatment of wounds, constituting about 68% of the preparations. This is followed by stem bark that constituted 19%, while the roots, bulbs and corms made up 13% of the
herbal preparations. In all cases, the treatment of wounds involved the external application of the medicine. No internal use was reported (Grierson and Afolayan, 1999).

A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolite. Some of these secondary metabolites are produced for self defense (Evans et al., 1986). Such metabolites are divided into different categories based on their mechanism of function i.e. bactericidal, antimicrobial, chemotherapeutic and bacteriostatic (Purohit and Mathur, 1999).

The use of and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists and natural-products chemists are combing the earth for phyto-chemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Lower plants belonging to the Leguminosae are abundant in flavonoid phytoalexins, which include flavanone derivatives structurally analogous to the anti-MRSA flavanones. In Asia, America and Africa the extensive use of natural plants as primary health remedies, due to their pharmacological properties is quite common (Conco, 1991). Pharmaceutical companies have spent a lot of time and money developing natural products extracted from plants, to produce more cost effective remedies that are affordable by the population (Farnsworth, 1994).

The different systems of medicine practiced in India. Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases. Most of these medicinal plants have been identified and their uses are well documented. But the efficacies of many of these plants are yet to be verified. In recent years, multiple resistance in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Marchese and Shito, 2001; Poole, 2001) have forced scientists into looking for new antimicrobial substances from various sources like medicinal plants.
Seventy-three species belonging to 34 plant families were reported being used as wound healing remedies by the traditional healers in Dogonland, the most common plant families being Fabaceae (17 plants) and Combretaceae (6 plants). The plants are used as first aids, in the washing of wounds, extraction of pus, as coagulants as well as for infected wounds. Leaves and roots are the most frequent plant parts used, constituting about 22 and 24% of the preparations, respectively, followed by stem bark and fruits (12% of each) (Inngjerdingen et al., 2004). Plants used for the treatment of wounds can have different properties like, anti-inflammatory, anti-microbial, healing, analgesic, haemostatic and immuno-modulating activities (Da Silva and Parente, 2001; Diallo et al., 1996).

Grinelle and Zhu (1994) investigated some South African plants, used for typical wound healing for their potential antibacterial activity since microbial infections can hamper the healing process. The effectiveness of South African traditional remedies against Gram-positive and Gram-negative microorganisms, only three publications could be found where the antibacterial activity of remedies used specifically for the treatment of wounds has been determined (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999; Kelmanson et al., 2000).

2.8.1 Phenolic compounds

Arogba, (2000) investigated that the composition of polyphenols (tannins) and the properties of an associated enzyme, polyphenol oxidase in the Nigerian mango kernel. 6.4% gravimetric yield of tannin from dry mango kernel meal contained tannic acid, gallic acid and epicatechin. Puravankara et al., (2000) reported that the presence of at least six phenolic compounds and eight phospholipids from mango seed kernels confirmed by chromatographic techniques. The phenolic preparation contained 9.6 mg %, water extracted phenolics, 69.5 mg % total phenolics and 6.39 mg % phospholipids. The phospholipids preparation contained 155.8 mg % phospholipids, 0.11 mg % water extractable phenolics and 0.19 mg % total phenolics.
Murga et al., (2000) studied the phenolic compounds from grape seeds, among them some low polymerized proanthocyanidins, their main monomer units, (+) catechin and (-) epicatechin and some low molecular phenolic compounds like gallic acid. Also studied that the HPLC coupled with two types of detectors, diode array and mass spectrometry was use for tentative identification and quantification of complex phenols and tannins in the extracts.

Various studies indicated that mango seed kernels contain different phenolic compounds and stable fat rich in saturated fatty acids so that it can be a good source of natural antioxidants (Parmar and Sharma, 1984; Nunez-selles, 2005). The Nigerian mango kernel has a good source of high quality fat and protein as well as tannin (Arogba, 1997). Mango seed kernel has potent antioxidant activity, high phenolic contents and also referred to be a good source of polyphenols, phytosterols as compesterol, β-sitosterol and tocophenols (Soong Barlow and Perera, 2004).