CHAPTER 1

INTRODUCTION

The *Staphylococci* are a group of Gram-positive bacteria, 14 species are known to cause human infections but the vast majority of infections are caused by only three of them. They are *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Of these single most important species is *Staphylococcus aureus*. Its main habitats are the nasal membranes and skin of warm blooded animals (Makoto *et al.*, 2001).

*S. aureus* is grouped with *Bacillus* sp on the basis of ribosomal RNA sequences. This immobile *coccus* grown in aerobic and anaerobic conditions, in which it forms grape-like clusters. Its main habitats are the nasal membranes and skin of warm-blooded animals, in which it causes a range of infections from mild, such as skin infections and food poisoning, to life threatening, such as pneumonia, sepsis, osteomyelitis and infectious endocarditis (Projans and Novick, 1997). The organism produces many toxins and is highly efficient at overcoming antibiotic effectiveness. In 1961, it developed resistance to methicillin, invalidating almost all antibiotics including the most potent β-lactams (Jevons, 1961)

Modern medium faces a crisis as new strains of multidrug resistant bacteria, threatening advanced treatments and intensive care. Every year nearly five million people die due to infections that do not respond to antibiotics particularly multi drug resistant *Staphylococcus aureus* (MRSA) infection. Drugs, which have kept as safe were begun to fail because bacteria are developing the ability to resist antibiotics.
The application of more antibiotics against these bacteria has developed multi drug resistance mechanism.

In 1942, the year that Penicillin G was introduced, resistant strains of *S. aureus* was found. In the next decade, some strains of *S.aureus* became resistant to olaendomycin, leucomycin, choloromphenicol, erythromycin and the tetracyclines. In 1961, Mitsuhashi *et al.*, isolated tetracycline resistant *S. aureus* from clinical sources.

Jevons 1961 in Great Britain, isolated coagulase positive methicillin resistant *S.aureus*. Methicillin destruction by these resistant strains was later demonstrated by Eriksen and Ericksen in 1963. Vancomycin was the only antibiotic used against it, but 1997, a Vancomycin resistant *S. aureus* (VRSA) was isolated (Makoto *et al.*, 2001).

*S. aureus* has emerged over the past several decades as a leading cause of hospital and community acquired infections (Lowy, 1998). A significant component in the “success” of *Staphylococcus aureus* has been its acquisition of antibiotic resistant factors (Chambers, 2001). As new antibiotics have come into use, *S. aureus* has responded soon after with resistant strains. This phenomenon has made therapy of *Staphylococcal* diseases a global challenge. Penicillin-resistant strains, for example, appeared in hospitalized patients within a short time after the introduction of the antibiotic: over time, penicillin-resistant strains has spread into the community to the extent that penicillin is now only of very limited value as treatment for *S. aureus* infections. There is a concern that methicillin-resistant *S. aureus* (MRSA) may be following the same path from the hospital to the community (Chambers, 2001). Accordingly, there is considerable epidemiological interest in the tracking of
strains to gain a more complete picture of the distribution of strains in the population and the dynamic of clonal spread (Crisostoma et al., 2001).

Since the 1970s, methicillin-resistant *S. aureus* (MRSA) has become the main cause of nosocomial infection worldwide. In 1997, a vancomycin-resistant *S. aureus* (VRSA) was isolated (Hiramatsu et al., 1999). We are now exposed to the threat of MRSA without having developed any antibiotics with greater activity than vancomycin. What is urgently needed is an insight of the mechanism by which the organism generates such a variety of toxins and develops resistant to so many antibiotics. One way in which to do this is to study its genome.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first isolated in England in 1961 shortly after the development of methicillin, The first penicillinase resistant semi-synthetic penicillin (Jevons, 1961). Since then, MRSA has become the most prevalent pathogen causing hospital infection throughout the world, and MRSA incidents are still increasing in many countries (Ayliffe, 1997). MRSA is resistant to practically all β-lactam antibiotics, a class of antibiotics represented by penicillins and cephalosporins (Chambers and Neu, 1995).

MRSA typically causes infections in inpatients, who have risk factors associated with health care. In the past 5 years, however, MRSA infections have been described in the general population. These infections arise in the community, and thus affected people lack traditional risk factors such as recent admittance, surgery, or long term residence in care facilities. Community-acquired MRSA infections can cause serious as well as fatal infections in otherwise healthy hosts (Naimi et al., 1998).

After more than 5 years of clinical usage, relatively few infections due to methicillin-resistant *Staphylococci* (coagulase-positive) have been reported
Coagulase-negative *Staphylococci* have been found to be resistant to methicillin, but these organisms are much less virulent than coagulase-positive strains. Jevons (1961) and Jevons, Coe and Parker (1963) in great Britain, noted an increase of coagulase-positive, methicillin-resistant *Staphylococcal* isolated from 0.55% in 1960 to 0.8% in 1962, but the clinical significance of the resistant strains was not known in detail. Barber and Waterworth, (1962) found a higher incidence (2.2%) at the Hammersmith Hospital, London, but these strains caused few significant infections. However Stewart and Holt (1963a) described an endemic methicillin resistant strain, which was isolated from 37 persons and caused one death in a British hospital.

Since 1981, strains of MRSA causing severe infections have been isolated from hospital patients in many countries including Australia, Ireland, England and the USA (Beard-Pegler *et al.*, 1988). In Royal Prince Alfred Hospital (RPAH) five prevalent strains of MRSA have been isolated. Epidemiological studies have shown that strains of two of these phage-typing patterns have been consistently isolated from general epidemics in many wards in RAPH and from several other hospitals in Sydney and NSW (Vickery and Beard-Peeers, 1986).

Seventy-one MRSA strains collected between January to March 2000 from patients from various wards in Hospital Universiti Kebangsaan Malaysia (HUKM), most of which were isolated from ICU, surgical wards and medical wards (Alfizah *et al.*, 2002).

For more than 30 years, vancomycin has been a reliable treatment for gram-positive bacterial infection. In Japan, injectable forms of Vancomycin were introduced in 1991, and have been used extensively for MRSA infection. Nevertheless, the mortality rate due to MRSA infection changed little with the
introduction of vancomycin in Japan (Hiramatsu et al., 1995). According to a 1995 nation wide survey of Vancomycin efficacy, therapeutic failure occurred in 21.3% of 845 MRSA pneumonia cases, while MRSA infection persisted in as much as 35.8% of patients after therapy for lower respiratory tract infections (Shimada et al., 1995). In recent years, S. aureus clinical isolated with resistance to teicoplanin, a glycopeptide antibiotic closely related to Vancomycin have been reported (Kaatz et al., 1990; Brunet et al., 1994). Subsequently VRSA were isolated from a Japanese surgical patient with a wound infection was unexpected (Hiramatsu et al., 1997).

At this juncture, there is an urgent need to find a remedy for these multidrug resistant S.aureus strains. Azardicta indica juss, Duranta pulmeneri jasq and Punica granatum Linn extracts could have the active principle against these MRSA strains. Using the above mentioned plant extracts study was conducted with the following aim and objectives. The study was suitably entitled as “MICROBIOLOGICAL AND BIOTECHNOLOGICAL STUDIES ON METICILLIN RESISTANT Staphylococcus aureus ISOLATES FROM TAMILNADU, INDIA.”.

**AIM OF THE STUDY**

1. To perform isolation and identification of Methcillin resistant S.aureus (MRSA) from suspected cases of different districts in Tamilnadu.

2. To conduct pathological and histopathological studies on MRSA.

3. To perform antigenic study by SDS-PAGE technique for identification of protein antigens.

4. To conduct a Western/Immunblot study for the confirmation of the antigens’ Immunogenic nature.
5. To perform FIGE - REA technique for identification of gene level variation.

6. To study mec-A gene in detail by PCR technique.

7. To construct the phylogenetic tree using 16S r RNA amplification test.

8. To sequence the whole genome of the potentially pathogenic strains.

9. To isolate and identify active principle of antibacterial compounds from plants *Azardicta indica juss*, *Duranta pulmeneri jasq* and *Punica granatum Linn.*