Chapter 1
Introduction
1. INTRODUCTION

Staphylococcus aureus is an opportunistic human pathogen capable of causing wound infections. There are over 40 virulence associated genes among S. aureus many of which are encoded by mobile genetic elements (Stott, 2005). Open wounds, particularly when they are chronic, provide a portal of entry for MRSA to the underlying tissues, which can lead to local or generalized infection. The presence of wounds in geriatric patients is a risk factor for MRSA colonization site (Guillaume et al., 2000).

MRSA were first reported in 1961 in Europe. CA-MRSA (Community Associated MRSA) infections were first reported in the late 1980s and early 1990s. MRSA is neither more infectious nor more virulent than Methicillin susceptible S. aureus (MSSA). It is difficult to control because of its resistance to antibiotics (Ashok et al., 2004).

The first reports of Vancomycin resistance in Coagulase negative Staphylococci was made in 1979 and 1983. In 1995, the first clinical isolate was reported in French child who received vancomycin for an MRSA line infection. In 1996, a wound infection caused by VISA was reported in Japan in a child receiving Vancomycin for an MRSA wound infection (Arjun Srinivasan et al., 2002). Oxacillin resistant S. aureus (ORSA) strains have become a widespread problem, which raises fears of increased therapeutic failures. Prevention of S. aureus infections and new treatments fare of utmost importance for the safety and treating an infection by cost effective means (Christof von Eiff et al., 2003).

First report of a Penicillin resistant strain of S. aureus was published in 1945, Penicillinase resistant penicillin was able to resist degradation by staphylococcal penicillinase is still being used as first line treatment. Methicillin was the first antibiotic in this class and was introduced in 1959. The first case of MRSA was reported in
England in 1961. Worldwide, an estimated 2 billion people carry *S. aureus*; of these, up to 53 million (2.7% of carriers) carry MRSA (Islam *et al.*, 2008).

In the mid-1980s, the β-lactamase stable penicillin resistant *S. aureus* had emerged and these strains are represented as MRSA. Use of Oxacillin as an alternative to Methicillin resulted in ORSA. Methicillin resistance in *S. aureus* is primarily mediated by *mecA* gene (Sridhar Rao, 2009). In 1963, the first nosocomial epidemic MRSA isolated from an infant treated with Penicillin. The first three MRSA were among 5,440 strains screened for Methicillin resistance. Their Minimum inhibitory concentrations (MICs) ranged from 3.1- 25 µg/ml. Resistance to Methicillin was heterogeneous and homogenous strains are highly resistant (Chambers, 1988).

**PREVALENCE OF MRSA IN OTHER COUNTRIES**

**MRSA IN MALAYSIA**

MRSA was reported in Malaysian hospital in 1972. It’s predominant in surgical wound infections (4.6%- 54.4%) (Arti Tyage *et al.*, 2008). Only minority (10^{-3}-10^{-5}) of MRSA cells express the resistant phenotype unless resistance is selected by prior antibiotic exposure. Low-level colonization was missed because of the presence of a mixture of MRSA with Methicillin susceptible strains (Van Enk and Thompson, 1992).

**MRSA IN SWEDEN**

MRSA during the last 3 decades, evolved as important causes of hospital infections worldwide. The prevalence of MRSA is still low in comparison with other European countries. 39 MRSA with diverse genetic backgrounds were identified as *S. aureus* on CHROMagar and *S. aureus* ID media. A combination of Cefoxitin disk and *S. aureus* ID was found suitable for rapid MRSA screening (Longzhu chi *et al.*, 2000).

**MRSA IN CANADA**

MRSA was first reported in Canada in 1981. National surveillance for MRSA was started in Sentinel hospital participating in Canadian Nosocomial Infection Surveillance Program (CNISP). From 1995-1999 the number of participating sites increased from 22-
34. Of 3,009 MRSA cases, 86% were acquired in a hospital, 8% were acquired in a long term care facility and only 6% were acquired in the community (Simor et al., 2001).

**MRSA IN FRANCE**

30-40% of *S. aureus* strains are Methicillin resistant and incidence of MRSA is 0.8 per 1000 hospital-days. Clinical MRSA ranges from 0.04-3.6 per 1000 hospital-days. Before 1990, homogeneously resistant MRSA to methicillin, aminoglycosides, fluoroquinolones and macrolides were found (Michelle Thouverez et al., 2003).

The clinical strains resistant to glycopeptides were impossible to treat. Its prevalence is highest in France among the European Union (Patrica Minary-Dohen et al., 2003).

**MRSA IN BRAZIL**

MRSA was first reported in Brazil in 1998. Staphylococcus infection can frequently occur in inpatients and lead to dire consequences. The frequency and role in nosocomial infection has been greater than in other countries. Few MRSA isolates were obtained from numerous geographical locations (Trindade et al., 2003).

**MRSA IN JAPAN**

MRSA since the 1980s has led to the popular use of glycopeptides in clinical practice for more than 20 year. The first report of Vancomycin intermediate *S. aureus* (VISA) for more than 20 cases have been reported. Three isolates of VRSA with (MIC) ≥32 mg/l) had been reported since 2002 from the US. Prudent use of Vancomycin as well as the development of alternative therapeutic options against MRSA is required to prevent the emergence of vancomycin non-susceptible *S. aureus* (Ji-Young Lee et al., 2005).

**MRSA IN LONDON**

NHS Trusts (Strategic Health Authority) varies with the region, ranging from eight in the North East region to 32 in London. The highest resident population for 2003 was 8,080,280 in the South East and the lowest 2,539,363 in the North East. There is considerable variation across the regions in reports of the rates of MRSA per 1000 bed
days made in the fourth year. The highest rates of 0.20/1000 bed-days, reported in fourth year of the scheme were from London region (Annual report of the regional and national analyses of the S. aureus surveillance scheme in England: April 2001 to March, 2005).

**MRSA IN AUSTRALIA**

CA-MRSA has emerged along the eastern seaboard of Australia, with the South West Pacific strains, Sequence Type 30 (ST30), predominantly affecting individuals of Polynesian descent, and the Queensland clone (ST93) affecting white people, which is not the community based study. Collection of data is critical for healthcare policies pertaining to empiric antibiotic use, for staphylococcus infections (Susan Vlack et al., 2006).

**MRSA IN NEW ZEALAND**

The first countries to experience CA-MRSA. The epidemiology changed at the beginning of the 2000s with the introduction of the British healthcare facility associated EMRSA-15 strain. By 2002 EMRSA-15 was common and between 2001 and 2007 MRSA were isolated equally in community and begun to increase (Alice Richardson et al., 2009).

In 1970s MRSA has become a nosocomial problem and sensitive to Clindamycin, Macrolide, Tetracycline, Trimethoprim- Sulfamethoxazole and Quinolones, or it may be resistant to all antibiotic except Vancomycin. No strains of S. aureus have failed to respond to Vancomycin. In May 1997, the Center for Disease Control and Prevention (CDC) confirmed that the first failure of Vancomycin in Japan. In 1998 at New York a man died of VRSA (Hakim et al., 2007).

Hospital-acquired MRSA cause surgical site infections to invasive disease. CA-MRSA strains have caused disease, including severe sepsis and pneumonia, in other body system. The community isolates of MRSA differ from their hospital counterparts in their demographic, clinical and molecular characteristics. The treatment of bacterial infections is complicated by the ability of bacteria to develop resistance to antimicrobial agents (Fenfang Li et al., 2005). Early detection of emerging trends in antimicrobial resistance may facilitate implementation of effective control measures (Sudha et al., 2001).
Emergence of CA-MRSA is a major threat with several important clinical implications: 1) Treatment failure with accompanying complications; 2) Infections caused was difficult to manage or expensive to treat; 3) The increasing prevalence will inevitably increase vancomycin use, (Chambers, 2001). CA-MRSA is more sensitive to multiple anti-staphylococcal agents such as Clindamycin, Erythromycin, Trimethoprim- sulfamethoxazole, aminoglycosides and Fluoro-quinolones (Schlesinger et al., 2003). The overall prevalence of MRSA infections has increased dramatically and the carriage rate is rising in medical and community (Lema et al., 2005).

The cell wall thickness and the increased production of nonamidated muropeptides may contribute positively to Vancomycin resistance in \textit{S. aureus} (MU50) by increasing the efficiency of affinity trapping and clogging of the mesh of the peptidoglycan outer layers. After the cell wall synthesis in the absence or presence of glucose and glutamine, different cell wall thicknesses and cross linking was prepared. Affinity trapping of vancomycin molecules by the cell wall and clogging of the outer layers of peptidoglycan by bound vancomycin molecules were the mechanism of Vancomycin resistance. The reduced cross linking and the increased affinity of binding to Vancomycin of the Mu50 cell wall presumably caused by the increased nonamidated muropeptides may also contribute to the resistance (Longzhu Cui et al., 2000).

Infections caused by \textit{S. aureus} used to respond to \textit{β} lactam and related group of antibiotics. Indiscriminate uses of multiple antibiotic, prolonged hospital stay, intravenous drug abuse, carriage of MRSA in nose are risk factors for MRSA acquisition. Burns and Orthopedics are risk where patients are on multiple antibiotics instead of Teicoplanin and Vancomycin (Vidhani et al., 2001). Multidrug resistant (MDR) \textit{Staphylococcus} isolates in hospital have been recognized as challenge in the hospital infection (Majumder et al., 2001). These strains are not only resistant to multiple antibiotics and act as a reservoir for drug resistant gene (Jessen et al., 1969). Approximately 30 cases of MRSA skin infection that occurred among HIV positive men were reported to the Los Angeles Country during October- November 2002. The hospital reference laboratory performed PFGE on 10
isolates from the index clinic and found that 8 isolates matched and 1 differed by 1 band, indicating predominant clonal MRSA strain (Lee et al., 2005).

*S. aureus* possess additional structured that resist the host defence system to succeed in an infection. The body fluids and tissues of animals naturally contain a variety of anti-microbial substances. One of the most important and widespread compounds of the constitutive defence systems is lysozyme, muramidase that cleaves peptidoglycan between the glycosidic beta-1, 4-lined residues of *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) (Agnieszka Bera et al., 2005). MRSA strains are genetically heterogenous and are resistant to Methicillin and β-lactam antibiotics (Baddour et al., 2006). The emergence of multi-drug-resistant strains and the indiscriminate use of antibiotics highlight the urgent need for development to treat bacterial infections. Peptides from marine invertebrates are new antibacterial agents due to their broad antimicrobial spectrum, highly selective toxicities, and inability of bacteria to develop resistance (Cueto et al., 2001).

The world’s oceans covering more than 70% of the earth’s surface, represents an enormous resource for the discovery of potential chemotherapeutic agents. Secondary metabolites are the products of biosynthetic pathways. About 100,000 secondary metabolites of a molecular weight below 2,500 have been characterized among approximately 50,000 marine microbial sources (Emad Abd El-Moniem Abada, 2008). Antibiotics isolated from marine sponge were active against human pathogens or marine bacteria. The diffusion of antibiotic agents in the sponges increases the efficiency and had defense against microbial infections (Dovi Kelman et al., 2001).

Sponges were obtained from the deepest ocean to the edge of the sea. There are approximately 15,000 species of sponges in the world, of which, 150 occur in freshwater, 17 are of commercial value. About 486 species of sponges have been identified in India. They can survive in a variety of circumstance. In the Gulf of Mannar and Palk Bay a maximum of 275 species of sponges have been recorded. The rapid development of the pharmaceutical market has brought about a bloom of information regarding various toxins native to the sponges (Boobathy et al., 2009b). Despite the wealth of biologically active...
secondary metabolites isolated from marine sponges, the potential functions of the compounds in antimicrobial chemical defense have rarely been explored. The first report of antimicrobial activity of sponge extracts was done by Nigrelli et al., (1959).

A critical issue in the drug development strategy for marine sponge is ensuring an adequate supply of compounds for clinical use while protecting the source organism (Pomponi et al., 1996).

The rich diversity in bioactive compounds from sponge has provided molecules that interfere with the pathogenesis of a disease. A combined approach of genetically modified bacterial fermentation followed by a limited number of chemical steps to produce molecules that are derived from sponge chemicals (Ravichandran et al., 2007).

Marine sponges are “gold mine” with respect to the diversity of their secondary metabolites discovered during the past fifty years. Of the 18,000 marine natural products, over 30% are from sponges. To increase the sponge biomass, knowledge is needed on the biosynthetic pathways and their regulation, which produce the metabolites (Marieke Koopmans et al., 2009). The marine sponges exhibit anti-bacterial, insecticidal, antiviral, antiplasmodial activities and antileishmanial activity (Lakshmi et al., 2009). The potential strategies to produce bioactive compounds from sponges include modification approaches in which large gene fragments responsible for production of bioactive compounds (Marieke Koopmans et al., 2009).

Recently various technologies developed to produce novel products from marine sponges; could contribute to human healthcare. More than 3,000 novel enzymes have been identified and these found their way in to biotechnological and industrial applications (Bertus Van den Burg, 2003). S. aureus produces a wide variety of extracellular proteins, several of which are lipolytic. Lipolytic enzymes role in disease is poorly understood, lipase appears to contribute to the localization of deep infection (Mark et al., 1992).

Marine biotechnology aims to develop methods for producing novel products originating from marine organisms to produce these products in sufficient amount to avoid over exploitation of marine natural resources (Rene et al., 2008).
Hence, the present study was focused to identify the compounds with bioactivity from marine organism against MRSA. The traditional medicines will not posses any side effects for the human beings. Wound healing is a complex process and efficient compounds were to be extracted for the treatment. Several advanced techniques were used to carry out for the final purification and structural elucidation of the compounds. The study was also focused on the designing of bioactive compounds from sponge, *Spirastrella inconstans* that could be active or efficient against MDR-MRSA isolated from the chronic wound among fishermans’ community by directly approaching them, since several studies were focused on the terrestrial isolate of MRSA. Marine sources such as enzymes produced from marine microorganism and protein from marine sponge serves as drug for several infection caused by pathogenic microorganisms, which were developing resistance.