Chapter 6
Summary
6. SUMMARY

Pus samples (n=92) were collected from the wound infection of fisherman community of East and West coastal area village regions such as Tamilnadu- Cuddalore, Devanampattinam, Pitchavaram, Chidambaram, Parangipettai, Kanyakumari, Nagercoil, Kollamcode, Collachal, Pudukkottai, Chennai - Mylapore, Ennore, Besant Nagar, Nochi Kuppam, Pudhupettai; Pondicherry - Periyakalapet, Kanagachettikulam, Veerampattinam; Kerala - Alapuzha and marine sediment and water samples from Gulf of Mannar and Rameswaram; Goa - Dona paula. Totally 100 Methicillin Resistant *Staphylococcus aureus* (MRSA) were isolated from several isolates of *Staphylococcus aureus* obtained from 92 pus samples and confirmed by using routine laboratory techniques. The epidemiological data showed that males were more prone to infection and with regard to age group adults were more prone to infection.

The antibiotic pattern of these isolates were studied by using twenty four antibiotic disks which includes, Amikacin (Ak)-30 mcg, Amoxicillin (Am)-10 mcg, Ampicillin (A)-10 mcg, Ceftizoxime (Ck)-30 mcg, Ceftazidime (Ca)-30 mcg, Co-Trimoxazole (Co)-25 mcg, Chloramphenicol (C)-30 mcg, Cefazolin (Cz)-30 mcg, Cephoxitin (Cn)-30 mcg, Clindamycin (Cd)-2 mcg, Ciprofloxacin (Cf)-30 mcg, Erythromycin (E)-15 mcg, Gentamicin (G)-10 mcg, Kanamycin (K)-30 mcg, Methicillin (M)-5 mcg, Moxalactum (Mx)-30 mcg, Nalidixic acid (Na)-30 mcg, Netilmicin (Nt)-30 mcg, Norfloxacin (Nx)-10 mcg, Oxacillin (Ox)-5 mcg, Penicillin G (P)-10 mcg, Rifampicin (R)-30 mcg, Tetracycline (T)-30 mcg, and Vancomycin (V)-5 mcg. Out of 72 MRSA isolates possessing more than 50% resistance. The DNA was extracted from all the isolates and only very few strains posses plasmids due to multidrug resistant gene lies on chromosomal DNA.
This study deals with Community acquired Methicillin Resistant Staphylococcus aureus (CA-MRSA) from fisher man (nature of resistance varies in marine environment). The bioactive compounds were necessary for the treatment of the chronic wound. The bioactivity was much more prevalent in sponge compared to that of other marine organism. Thus marine organism, particularly (sponge) Spirastrella inconstans was selected, which was collected from the deep sea. The sponge powder was taken for solvent extraction method to detect antimicrobial activity using selective solvents such as Chloroform, Dichloromethane, Methanol, Ethanol, Petroleum ether and Acetone, among which Chloroform was effective and inhibits MRSA with maximum activity.

The protein was fractioned from the powdered form of Spirastrella inconstans by ammonium sulphate precipitation method and those fractions (20%, 40%, 60% and 80% of saturation level) were purified using dialysis. About 1000 μl of dialysate were loaded on Sephadex G-50 chromatographic column and purified fractions were collected at a rate of 13 drops/min, in which 75 fractions for each percentage of saturation was collected (totally 300 fraction). Amount of protein (Maximum absorbance for all the fractions) content was estimated in UV-Vis Spectrophotometer by calculating absorbance Vs Protein content. It was also quantitatively estimated by Lowry’s method. Preliminary detection of compound was carried out by paper and Thin layer Chromatography (TLC). Rf values were calculated to detect the type of amino acid, using standard amino acid. Those fractions with same compound were mixed together and the efficient fractions were pooled out among all the fractions obtained.

The Spirastrella inconstans protein content of only 2 fractions (fraction bearing maximum protein content by elution) showed better result in SDS-PAGE (Sodium Dodecyl Sulphate Polycrylamide Gel electrophoresis) compared to that of other 300 fractions. The molecular weight was calculated as 3,000 Da i.e., equal to that of insulin. As insulin can be used for the treatment of wound infection from olden days. These proteins were to be purified further for the drug discovery and molecular structural elucidation of protein molecules.
Marine bacterial isolates from different marine sources were isolated and the enzymes were produced such as amylase, DNase, esculinase, lipase, gelatinase and alginate using differential media. The extracellular enzymes were tested against Methicillin Resistant *Staphylococcus aureus* (MRSA) and the enzyme from the rat fish isolate was more effective compared to others. But compared to that of sponge protein, marine bacterial enzymes were not much more effective. Thus the bioactive compounds from the marine organisms were tested against Multi drug -Methicillin resistant *Staphylococcus aureus* (MD-MRSA).

Minimal Inhibitory Concentration (MIC) using microtitre assay were performed, to detect the lowest concentration of *Spirastrella inconstans* required to inhibit MRSA. Dosage level of sponge extract was estimated by TLC. Lysostaphin was extracted from *Staphylococcus stimulans* for lysis of cell wall of MRSA to isolate the chromosomal DNA, for further characterization of resistance gene location.

Lectin (type of protein) from sponge was characterized for inhibition of MRSA. Protease from fish waste, which has the ability to destain blood clot, was used for further characterization and to detect antimicrobial activity. All the enzymes were studied by assay procedure (particular unit of enzyme produced by the organism in particular time) for detecting best enzyme by molecular level. Lipase showed better result compared to others. The molecular weight of the compound was detected by SDS-PAGE as 3,000 Da.

IR Spectrum were recorded on a NICOLET 360 FT-IR spectrophotometer. Absorption value obtained by UV spectrum using TECHCOMP 8500 spectrophotometer was 1.7141. Wave length obtained by HPLC analysis was 254 nm. SEM result showed that at X 270 illumination 50 µm, at 2,300 X illumination 10 µm, at 6,000 X illumination 2 µm, at 2,700 illumination 5 µm. Thus the bioactive compound was identified as Glutamic acid by structural elucidation using $^1$H and $^{13}$C NMR from the marine source against dread full wound caused by MRSA among fisherman community.
Although substantial progress has been made in identifying novel drug leads from the sponge’s resources, great efforts are still needed to advance to clinical applications. The grinded colorless parenchyma of *Spirastrella inconstans* in a blender was centrifuged to remove the fibers and supernatant was used in lyophilized form. It can be administrated twice a day by applying tropically on the wound surface of community people. The identified compound is purified form of Glutamic acid from *Spirastrella inconstans* protein, which in lyophilized form can favor for the healing of tendon lesions and of wounds by acting against MRSA. Glutamic acid compound can be helpful in the treatment of arthritis, autoimmune diseases, fibrosis, wound infection, intestinal disorders such as ulcerative colitis, peptic ulcers, and connective tissue diseases.