Chapter 2
Review of Literature
The prevalence of MRSA strains in wound infection among the fishermen was due to the virulence factors. As MRSA develops resistant to increasing numbers of antibiotics, the remaining effective antibiotics are used often - increasing the selection pressure to become resistant. The discovery of new secondary metabolites derived from marine bacteria and *Spirastrella inconstans* were effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), although a compound from organism of terrestrial region differs from marine region. The diversity of sponges has been demonstrated by continued discovery of novel compounds, having pharmacological properties.

Mates and Sudakevitz, (1972) suggested that *Staphylococcus aureus* produces an extracellular lipase in a synthetic medium. The composition of the medium affected the amount of enzymes produced: medium containing 2% of peptone was optimal for enzyme production. Tweens added to the media decreased the amount of lipase synthesized depending on the concentration used and the nature of the fatty acid bound to the molecule of polyoxyethylene sobitan. Carbohydrates that the bacteria could metabolize inhibited lipase production. Bile salts and NaCl decreased the amount of enzyme produced. Optimum lipase production was at pH 7.5 and 37°C.

Lawrence Drew *et al.*, (1972) reported that Kirby-Bauer disc diffusion method for susceptibility testing may fail to detect all Methicillin resistant strains of *S. aureus*, largely because such strains are remarkably heterogenous. The disc diffusion technique of Bauer *et al.*, 1972, is reliable for the detection of *S. aureus* resistant to Methicillin and related to antimicrobials when incubated at 35°C.

Zuccarelli *et al.*, (1990) noted that nosocomial infections caused by MRSA are a significant epidemiological problem. Detecting the sources of epidemic strains and
preventing their access to patients, however, depend upon the availability of techniques to reliably distinguish among MRSA strains. Restriction enzyme analysis of plasmid DNA for use as an epidemiological marker of MRSA strains. The diversity of plasmid types were assessed by examining 120 clinical and environmental MRSA isolates from five southern California hospitals and from the ATCC. 37 distinctive EcoRI digestion patterns were observed. Few isolates (4.2%) lacked plasmids, and some (6.7%) contained DNA that were not digested by EcoRI. Several isolates (12.5%) contained two or more plasmids. Diversity and stability of plasmid profiles provide an effective means for discriminating between strains.

Jaime Fabregas et al., (1991) reported that the antibacterial substance producing bacteria from seawater, sediments, and extraction activity with different organic solvents were carried out, from eight strains of bacteria only fifteen were found to have antimicrobial activity, some against Pseudomonas sp. which were highly resistant to current antibiotics. The Corynebacterium- Arthobacter group accounted for 30.7% of the strains isolated and 46.7% of those with antimicrobial activity. Only one of the fifteen antimicrobial strain produced pigments. Productions of antimicrobial substances were varied with the culture medium and the screening test used (disc and plate diffusion). Of three organic solvents of differing polarity used sequentially to extract the active principles from the 15 active strains, ethyl acetate was the most efficient.

Jan Rollof and Staffan Normark, (1992) justified that the S. aureus lipase gene encodes 76 kDa protein. Extracellular lipase purified from culture supernatants is only 45-46 kDa. The lipase is secreted in vivo as 82 kDa protein with enzymatic activity. It is then sequentially processed, both in culture and in cell free supernatants. Protein sequencing demonstrates that the N-terminal region of the 82 kDa prolipase, comprising 295 amino acids were cleaved from the central and C-terminal moieties, which contains the active site. A metallo cysteine protease is probably responsible for initiating this processing. The hydrophobic, mature lipase is resistant to further protease degradation and retains the full catalytic activity of the prolipase.

McClintock and Gauthier, (1992) measured the Methanol toluene extracts of 17 common Antarctic marine sponges collected from shallow waters in McMurdo Sound in
October-December 1989 and were tested for suppression of growth of gram-positive and negative bacteria. Weak to moderate levels of antimicrobial activity occurred in all sponges. Antimicrobial activity were more common when gram-negative bacteria were exposed to sponge extracts; 47% of the sponge extracts caused growth inhibition in one or more gram-positive bacteria, while 100% of the extracts caused growth inhibition in gram-negative bacteria. Particularly strong activity were observed against two species of gram-positive bacteria exposed to extracts of the sponge *Latrunculia apicalis* and against one strain of gram-negative bacterium exposed to extracts of the sponge *Haliclona sp.* Antimicrobial activity in these polar sponges is widespread but generally weaker than that found in temperate and tropical sponges.

Concepcion *et al.*, (1994) measured the activity in terms of zone of inhibition increases from crude extracts to Kupchan extracts to column chromatography fractions. Within the column chromatography factions there is a general decrease in activity when these fractions are further fractionated. Strong activity when these fractions are further fractionated. And third, strong activity and wide activity are generally found in the same extractor fraction. The results of rapid screens provide evidence for the potential use of marine natural products as source of novel antibiotics. Rapid screens that compare the activities of these ready made potential antibiotics with standard drug lead to the discovery of drugs having specific modes of action, a discovery that cannot yet be achieved efficiently by rational drug design. The antimicrobial assays done may confirm the hypothesis that marine sponges and tunicates are indeed potential sources of novel compounds with biomedical potential.

Pomponi *et al.*, (1996) pointed out the marine sponges are the source of many bioactive compounds with therapeutic potential. A critical issue in the drug development strategy for marine natural products is ensuring an adequate supply of compounds for clinical use while protecting the source organism and its habitat from over exploitation. One approach is the development of techniques for in vitro production of bioactive compounds. Replicative cultures of the sponge *Teichaxinella morchella* have been established. Technique for monitoring sponge cell responses to growth factors by semi automated micro titer plate assays were developed, including sulfo rhodamine B method.
for total protein. The identity of cell culture stimulated to divide by vertebrate growth factors and lectins were verified by the in vitro production of stevensine (odiline), a compound which is characteristic of *T. morchella*. Cultures retained their ability to synthesize stevensine after doubling, which demonstrates the feasibility of in-vitro production of bioactive, sponge derived natural products.

James *et al.*, (1996) isolated the biofilm forming marine bacterium, D2, isolated from the surface of the tunicate *Ciona intestinalis*, were found to produce a novel, 190-kDa protein with antibacterial activity. The protein with two subunits of 60 and 80 kDa, joined together by non covalent bonds, were shown to be released by D2 cells in to the surrounding medium during stationary phase. N-terminal sequence analysis revealed no close similarity of this protein to any other proteins within the Swiss Prot database. Bacteriocidal activities against a wide variety of marine and medical bacterial isolates were observed, 77% of the strains tested being sensitive to the protein. Bacterial strains vary in their resistance to the D2 proteins with D2 itself being among the most sensitive with an MBC in liquid suspension of 4 µg/ml. The ability of the D2 bacterium to produce an antibacterial factor in addition to its inhibitory effects on marine invertebrates and algae indicates that D2 has the potential to greatly affect the survival of the marine surface environments.

Newbold *et al.*, (1999) found that the secondary Metabolites produced by sponges have antimicrobial effects. Organic extracts from 33 species of Caribbean sponges were assayed for antibiotic activity against 8 strains representing 6 genera of Marine bacteria, opportunistic pathogen, fouling bacteria. The concentrations of extracts used were volumetrically equivalent to whole tissue concentration of sponge. Bio assay results revealed that 16 species exhibited antibiotic activity against atleast one bacterial isolate and two bacteria isolated from necrotic sponge tissues. Extracts from *Amphimedon* species, *Aplysina lacunose* and *Ptilocaulis spiculifera* exhibited the most antibiotic activities exceeding those of a control Gentamycin. Caribbean Sponge communities do not produce similar antibacterial or broad spectrum metabolites.

O’ Brien *et al.*, (1999) investigated that Western Australia has been able to prevent MRSA from becoming established in its hospitals. A single-strain outbreak of
MRSA occurred in metropolitan teaching hospital following admission of an infected patient from a remote community. The strains responsible for the outbreak were 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. Isolates were typed by resistance pattern, plasmid analysis, contour clamped homogeneous electric field electrophoresis, bacteriophage pattern, and coagulase gene restriction fragment length polymorphism. It were found that of the people carrying MRSA, 39% in the former community and 17% in the latter community were carrying an MRSA strain which were indistinguishable from hospital outbreak strain.

John Merlino et al., (2000) identified a new chromogenic plate medium, CHROMagar Staph aureus (CHROMagar, Paris, France) for the identification of S. aureus on the basis of colony pigmentation. The abilities of CHROMagar Staph aureus, thermostable nuclease (DNase), and mannitol salt agar (MSA) to indentify S. aureus isolates (n=114) and discriminate between S. aureus and coagulase- negative staphylococci (CoNS; n=22) were compared. CHROMagar Staph aureus proved to be more sensitive and specific than DNase and MSA. All CoNS encountered in this study with the exception of S. chromogenes could be easily differentiated from S. aureus on this medium. The supplementation with 4 µg of oxacillin or Methicillin/ml allowed simple identification of Methicillin resistance in hospital acquired S. aureus strains which show multiple drug resistance profiles. CA-MRSA showing non-multi-drug resistance profiles require further evaluation on this new chromogenic medium.

Chambers, (2001) confirmed that the genetics of Methicillin resistance is as complex as the biochemistry. Initial confusion about whether the genetic determinant, mec of Methicillin resistance resided on plasmid or chromosomal deoxyribonucleic acid (DNA) has been resolved. Evidence for a plasmid location was indirect and based on characteristics of elimination of mec from resistant strains. However, some conditions associated with the elimination, transduction, and transformation of mec suggested that the determinant were chromosomal. Transformation of mec by chromosomal but not
plasmid DNA and elucidation of its map location to the pur-nov-his gene cluster have conclusively demonstrated that the determinant is chromosomal.

Swenson et al., (2001) showed the inoculation methods to be used in Oxacillin screen test for *S. aureus*, were with plates containing 6 µg of Oxacillin/ml and 4% NaCl using different inoculation methods. The organisms selected for testing were 19 hetero resistant *meca* and 41 non- *meca* for which Oxacillin MICs were near the susceptible breakpoints. The best combination of sensitivity and specificity were 1-µl of 0.5 McFarland standards. Oxacillin screen medium that fail to grow hetero resistant strains can be detected by using *S. aureus* ATCC 43300 as a positive control in the test and by using transmitted light. Lack of specificity with commercial lots may be difficult to detect using any of the current quality control organisms.

Majumder et al., (2001) studied the prevalence of MRSA from a Referral hospital in Assam. Methicillin resistance among the *S. aureus* isolates were 52.9% and 15% among the Coagulase negative staphylococci. Resistance to all antibiotics tested among the Methicillin resistant and Methicillin sensitive staphylococci were found to be 23.2% and 6.6% respectively. Higher resistance to multiple antibiotics in Methicillin resistant strains as compared to Methicillin sensitive strains was found to be statistically significant. Ciprofloxacin resistance among the strains was still lower in comparison to the findings from other parts of the country.

Grisold et al., (2002) evaluated a molecular assay for the simultaneous detection of a *S. aureus* - specific gene and the *meca* gene, responsible for the resistant to Methicillin in staphylococci. The assay included an automated DNA extraction protocol conducted with MagNA pure LC benchtop instrument and real-time PCR conducted with a Light cycler instrument. The performance and robustness of the assay were evaluated for a suspension of MRSA strain with a turbidity equivalent to McFarland standard of 0.5. The specificity of the new molecular assay was tested with a panel of 30 gram-negative and gram-positive bacterial strains other than MRSA without any cross-reactivity. Among 109 MRSA investigated, all MRSA gave positive results for *S. aureus* - specific genomic target, and one were positive for *meca* gene. The molecular assays were suitable for routine molecular laboratory.
Michelle Thouverz et al., (2003) isolated 50 consecutive non-replicate MRSA isolates from 1999-2003. Susceptibility rates to Gentamicin, Tobramycin and Ofloxacin remained stable (95, 16 and 4%, respectively). In contrast, the proportion of MRSA susceptible to Erythromycin increased progressively from 10·5 to 32·5% (P, 0·001). Non-epidemic strains were more frequently susceptible to Ofloxacin (31·8 versus 1·1%) and Tobramycin (45·4 versus 16·8%) than epidemic strains; those isolates that were susceptible to all antibiotics tested belonged to sporadic clones. The increase of Erythromycin susceptibility within MRSA were caused by emergence of clone C. The balance between the selection pressure exerted by antibiotics and disadvantage of lower replication rates of resistant strains in absence of antibiotics complicates the biological model of clonal dissemination of epidemic MRSA.

Ridzwan et al., (2003) found that sea cucumber promotes wound healing and contains antibacterial, antioxidant properties and fatty acids. Relief from pain and irritation is one of the reported benefits in those taking sea cucumbers based remedies for the treatment of skin conditions. The antinociceptive agents found in water extracts and coelomic fluid from sea cucumbers, Holothiria leucospilota Brandt, Bohadschia marmorata vitiensis Jaeger and coelomic fluid from Stichopus hermanii were reported for pain relief, in arthritis and burning, wound healing and post surgery pain.

The existence of a tachylectin related protein in demosponge Suberites domuncula, termed Suberites lectin. The steady state level of expression of the Suberites lectin rises in primmorphs in response to lipopolysaccharide, an effect that were prevented by co-incubation with D-GlcNac. The natural sponge lectins were purified by affinity chromatography; it has a size of 27 kDa and displays antibacterial activity against the gram-negative bacteria E. coli and the gram-positive bacteria S. aureus. The putative protein, deduced from the cloned gene, is identical or similar to the purified natural protein, as demonstrated by immunological cross reactivity with specific antibodies. The S. domuncula lectin acts as an antibacterial molecule involved in immune defense against bacterial invaders (Schroder et al., 2003).

Hoq et al., (2003) collected hemolymphs from the mud-crab, Scylla serrata and were subjected to antibacterial assay to investigate antibacterial protein. Induction was
done by injecting *E. coli* ATCC 25922. The induced hemolymph showed antibacterial activity against a range of gram-positive bacteria including few antibiotic resistant strains, whereas the non-induced hemolymph were not appeared to be active against those tested. The induced hemolymph was subjected to SDS-PAGE and seven proteins were detected. The molecular weight ranges from 36 to 84.5 kDa. The proteins were fractionated by gel filtration chromatography. Repeated antibacterial assay of the chromatographic fractions against previously proved sensitive bacteria showed that only a few fractions were responsible for the activity and purified proteins showed better activity than crude hemolymph. Five proteins with molecular weights 64 kDa, 61 kDa, 56.5kDa, 49 kDa, and 36 kDa were present in all fractions.

Marshall *et al.*, (2003) estimated that about half of all *S. aureus* strains found in many medical institutions are resistant to Methicillin. The next decades should be focused in the development of alternative drugs and the recovery of natural molecules that would allow the consistent and proper control of pathogen that caused diseases. Ideally, these molecules should be a natural possible, with wide range of action over several pathogens, easy to produce, and not prone to induce resistance.

Adebayo Shittu *et al.*, (2004) reported the isolation, molecular identification and genotyping of multi resistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* from skin and soft tissue infections. Accurate identification of three coagulase-negative staphylococcal isolates was achieved using PCR, while the API STAPH method failed to identify an isolate of *S. haemolyticus* fully. The PCR assay detects polymorphism in the 16S-23S rRNA spacer region, is potentially useful for rapid and accurate identification of coagulase-negative staphylococci.

Methicillin (Oxacillin) resistant staphylococci (MRS) have emerged as major clinical and epidemiological pathogens, and there have been frequent reports of MRSA infections in the veterinary field. The MRSA Screen latex agglutination test was compared with an Oxacillin agar screen test, MIC determination, and *mecA* PCR assay, the “gold standard”. In an analysis of 15 *mecA*-positive and 48 *mecA*-negative *S. aureus* animal isolates, as well as 9 *mecA*-positive and 147 *mecA*-negative, coagulase-negative staphylococcal animal isolates, the latex agglutination test surpassed the widely used
oxacillin agar screen method and MIC determination, with a sensitivity and specificity of 100%. The MRSA-Screen tests were reliable and rapid method of detecting MRSA in the veterinary field (Lee et al., 2004).

The MRSA and Methicillin resistant coagulase negative staphylococci are the major nosocomial pathogens in Neonatal Intensive Care Units. *S. aureus* CONS were isolated from neonatal septicemic cases during October 2001 to August 2003, and tested for antimicrobial susceptibility using Kirby Bauer disc diffusion technique. Among 161 isolates of *S. aureus* and 118 of CONS, 39.1 and 21.2% respectively were resistant to Methicillin. More than 82% of MRSA were found to be resistant to Ciprofloxacin, Co-trimoxazole, Erythromycin, Vancomycin, Penicillin and 15% of MR-CONS were resistant to the antibiotic tested. More than 68% of MRSA and 80% of the MS-CONS were sensitive to Netilmicyn and Ofloxacin. However, no strain (MRSA and MR-CONS) were resistant to Vancomycin. Therefore, when antimicrobials other than Vancomycin are considered for therapy, *in-vitro* susceptibility test of every isolate of MRSA and MR-CONS is essential (Vinod Kumar and Neelagund, 2004).

Hong Young Yan et al., (2004) narrated that Taiwan situates in vary unique highly bio diversified location and has great biodiversity to offer many marine natural compounds for use in future development. However the limited manpower in identifying possible useful compounds hinders its long term potentials and development. Efforts from governmental agencies, research communities and private sectors need to be coordinated to make the best use of highly developed aquaculture technology in Taiwan to enhance in captivity mass production of raw materials needed for extraction of marine natural compounds which protect natural population and habitat from over exploitation.

Ashok et al., (2004) advised that in an case report obese lady aged 51 years were admitted in hospital with complaints of pain and swelling of abdominal wall, vomiting since three days and fever since 10 days. After one year of surgery, the patient developed wound dehiscence and discharge. MRSA were isolated from the wound, mesh, external nares and throat. Initially she were started on Clindamycin and discharged from the hospital. After 5 months, she came back to hospital with infection at the same site. The
patient was treated with Vancomycin and MRSA clearance, she responded to treatment with complete healing of wound and MRSA clearance.

Fridkin et al., (2005) found the MRSA infection from 2001 through 2002, 1,647 cases infection were between 8% and 20% of all MRSA isolates. The annual disease incidence varied according to site (25.7 cases per 100,000 population in Atlanta vs. 18.0 per 100,000 in Baltimore) and was higher among persons less than two years old than among those who were two years of age or older (95% confidence interval, 1.19-1.92) and among blacks than among whites in Atlanta (age-adjusted relative risk, 2.74; 95% confidence interval, 2.44 to 3.07). Six percent of cases were invasive, and 77% involved skin and soft tissues. The infecting strains of MRSA was often (73%) resistant to prescribed antimicrobial agents. Among patients with skin or soft-tissue infections, therapy to which the infecting strain was resistant did not appear to be associated with adverse patients reported outcomes.

Fenfang Li et al., (2005) evaluated the emergence of MRSA has generated concern among medical and public health professionals. Population based antimicrobial resistance surveillance system to examine epidemiologic trends for MRSA from outpatients and inpatients in Hawaii. Pediatric and adult patient were compared to assess characteristics of MRSA isolates specific for each group. From 2000-2002 8,206 (26%) of 31,482 total S. aureus isolates were MRSA. The proportion of MRSA isolates increased in both outpatient and inpatient clinical settings (p<0.01). When stratified by age, annual trends showed a significant increase in the proportion of MRSA in adult patients (from 24-30%, p<0.01) but not in pediatric patients (from 25-27%, p<0.05%). Although MRSA isolates from adults demonstrated high resistance to non-β-lactams, most MRSA isolates from pediatric outpatients remained susceptible to non-β-lactams.

A standardized protocol enabling rapid NMR data collection for high quality protein structure determination is presented that allows one to capitalize on high spectrometer sensitivity: a set of five G-matrix Fourier transform NMR experiments for resonance assignment based on highly resolved 4D and 5D spectral information is acquired in conjunction with a single simultaneous 3D \(^{15}\text{N},^{13}\text{C}_{\text{aliphatic}},^{13}\text{C}_{\text{aromatic}}\)-resolved [\(^1\text{H},^{1}\text{H}\)]-NOESY spectrum providing \(^1\text{H}-^{1}\text{H}\) upper distance limit constraints. The
protocol was integrated with methodology for semi automated data analysis and used to solve eight NMR protein structures of the Northeast Structural Genomics Consortium pipeline. The molecular masses of the hypothetical target proteins ranged from 9 to 20 kDa. Between 1 and 9 days of instrument time were invested per structure, which is less than ≈10-25% of the measurement time. The protocol presented here effectively removes data collection as a bottleneck for high throughput solution structure determination of proteins up to at least ≈20 kDa, while concurrently providing spectra that are highly amenable to fast and robust analysis (Gaohua Liu et al., 2005).

Shobha et al., (2005) showed a total of 205 samples from hospital personnel and environment were collected from casualty, oncology and multidisciplinary cardiac unit ward of Kasturba Medical College Hospital, Manipal. Isolates were identified and antimicrobial susceptibility test were performed according to standardized disc diffusion Kirby-Bauer method. Each of the isolates were screened for Methicillin resistance using Oxacillin disc on MHA plate followed by MIC for Methicillin and Cefoxitin susceptibility test by disc diffusion method. Sixty five out of 205 strains (31.7%) were Staphylococcus sp., and all of them were coagulase negative. Highest number of Methicillin resistance coagulase negative strains (3/9, 33.33%) were isolated from stethoscope of multidisciplinary cardiac unit ward followed by carriers in the anterior nares (2/9, 22.22%). Methicillin resistant Coagulase negative staphylococci are prevalent in anterior nares of hospital personnel and in the hospital environment thereby providing a definite source for hospital acquired infection. All isolates were sensitive to Vancomycin, Ciprofloxacin, and Amikacin.

Bhateja et al., (2005) used three methods to screen 160 S. aureus clinical isolates along with ATCC quality control strains. Subsequently, The MIC of all the 160 clinical isolates were <4 µg/ml determined by NCCLS methodology and were classified as Vancomycin susceptible by NCCLS criteria but 23 strains were positive by Hiramatsu method, two grew on MHA (5 µg/ml Vancomycin) while CDC method correctly identified no Vancomycin intermediate S. aureus (VISA) or Vancomycin resistant S. aureus (VRSA) strains with reference to MIC.
Antibacterial abilities of intestinal microflora of Atlantic salmon from the Zeimena River against the tested pathogenic bacteria ranged from 0-15%. Investigation revealed that the intestinal bacteria exhibited a relatively high antibacterial activity against *B. subtilis, C. albicans, E. coli, S. aureus*. The antibacterial activity of intestinal microflora was low in comparison with that of the cultured Atlantic salmon. This can be related to the closed water recirculation system, which creates better conditions for pathogenic bacteria. Hence, constant attack of pathogenic microorganisms contributes to the formation of fish intestinal microflora possessing antibacterial activity, which helps the organism to resist infection (Vesta Skrodenyte- Arbaciauskiene et al., 2005).

Ji-Young Lee et al., (2005) investigated in vitro activities of arbekacin-based combination regimens against Vancomycin hetero-intermediates *S. aureus* (hetero-VISA). Combination of Arbekacin with Vancomycin, Rifampicin, Ampicillin-sulbactam, Teicoplanin or Quinupristin-dalfopristin against seven hetero-VISA strains and two MRSA strains were evaluated by the time kill assay. The combinations of Arbekacin with Vancomycin, Teicoplanin or Ampicillin Sulbactam showed the synergistic interaction against hetero-VISA strains. Data suggest that these Arbekacin based combination regimens may be useful candidates for treatment options of hetero-VISA infections.

Li Zheng et al., (2005) isolated marine bacteria from seawater, sediment, marine invertebrates and sea weeds collected from different coastal areas of the China sea. Ethyl acetate extracts of bacterial fermentation were screened for antimicrobial activities by agar diffusion method. The results showed that 42 strains of isolates have antimicrobial activity. The proportion of the active bacteria associated with marine invertebrates (20%) and seaweeds (11%) is higher than that isolated from seawater (7%) and sediments (5%). The active marine bacteria were assigned to the genera *Alteromonas, Pseudomonas, Bacillus* and *Flavobacterium*. The TLC autobiographic overlay assay implied that the antimicrobial metabolites produced by four stains with wide antimicrobial spectrum were different. Due to competitive role for space and nutrient, marine bacteria associated with marine macro organisms (invertebrates and seaweeds) could produce antibiotic substances. These marine bacteria were expected to potential resource of natural antibiotic products.
Lema et al., (2005) revealed that MRSA have become more prevalent in community associated infections. CA-MRSA tends to be more susceptible than hospital acquired strains and therefore may be more of a challenge to identify in clinical laboratory. The ability of BD CHROMagar MSRA to detect CA-MRSA isolates, which is a chromogenic media with cefoxitin for selectivity. A mauve pigmented colony which grows within 24 hrs can be identified as MRSA. At 48 hrs, a slide coagulase confirms a mauve colony as MRSA. A well characterized set of 79 clinical S. aureus isolates and the USA 300 CA-MRSA strains were included in the evaluation. All isolates were recovered from patients hospitalized less than three days. The isolates were inoculated to CHROMagar MRSA as follows: From a fresh subculture on sheep blood agar, 0.5 McFarland suspension was diluted 1:100 and then 10 µL were inoculated to each plate. 77/80 isolates were detected as mauve colonies on the CHROMagar MRSA at 24 hrs. Both systems called one isolate oxacillin susceptible. BD CHROMagar MRSA successfully isolates and identifies CA-MRSA. This data supports the use of CHROMagar MRSA for nasal surveillance cultures which may contain either hospital or community associated MRSA.

Swenson et al., (2005) assessed the Cefoxitin disk diffusion test for predicting mecA- mediated Oxacillin resistance in staphylococci during different phase study. In phase 1, one laboratory tested 62 and 53 strains of S. aureus and (CoNS), respectively. These data were used to choose the provisional Cefoxitin DD breakpoints of ≤19 mm/≥20 mm for S. aureus and ≤24 mm/≥25 mm for CoNS for the next phase of testing. In phase 2, 10 laboratories each tested approximately 40 in-house strains of staphylococci using MHA. In this phase, the sensitivity and specificity, respectively, of the Cefoxitin disk test were 98 and 100% for S. aureus and 99 and 96% for CoNS. The Cefoxitin DD test performed equivalently to oxacillin broth micro-dilution (BMD) and to Oxacillin DD tests among S. aureus and mecA-positive CoNS strains but gave better results than Oxacillin BMD or Oxacillin DD for mecA-negative strains of CoNS. 61 challenge strains of CoNS for which the Oxacillin MICs were 0.5 to 2 µg/ml were tested in a laboratory to determine the effectiveness of the Cefoxitin DD test for this group of borderline resistant isolates. The Cefoxitin DD test is preferred over Oxacillin DD test for predicting mecA- mediated Oxacillin resistance in S. aureus and CoNS.
Mostafizur Rahman *et al.*, (2005) isolated a total of 28 *S. aureus* strains from skin lesion samples. Results of antibiotic susceptibility test showed they were resistant to Ampicillin (72%), Amoxycillin (72%), Penicillin (72%), Co-trimoxazole (15%), Cloxacillin (50%) Tetracycline (11%), Cephradine (22%), Cephalexine (7%) and Nalidixic acid (18%). To understand whether this drug resistant phenomenon was plasmid mediated or not, plasmids were isolated from a chosen strain of *S. aureus* (S$_2$) and 23 KB plasmid were obtained.

Desjardins *et al.*, (2006) performed rapid detection of MRSA by PCR directly from nasal specimens with the IDI-MRSA assay. To improve the efficiency of screening, it was evaluated the performance of the IDI-MRSA assay for the detection of MRSA from pooled and unpooled specimens cultured in a selective broth. Of the 287 specimens evaluated, 71 were culture and PCR positive, 203 were culture and PCR negative, 3 were culture positive and PCR negative, 8 were culture negative and PCR positive, and 2 remained inhibited. A Methicillin susceptible *S. aureus* isolate were recovered from five of the eight specimens with false-positive PCR results. Compared to the results of culture, the sensitivity, specificity, and negative and positive predictive values of the IDI-MRSA assay for detection of MRSA from broth were 96%, 96%, 90%, and 98%, respectively. Following implementation of the IDI-MRSA assay, PCR-positive broths were sub-cultured for evaluation of assay performance. Of the 298 IDI-MRSA assay-positive broths, the results for 103 could not be confirmed by culture. The positive predictive value of the IDI-MRSA assay fell from 90% during the evaluation phase to 65% post implementation.

Yhu-Chering Huang *et al.*, (2006) investigated that 50% to 80% of *S. aureus* isolates causing nosocomial infections in 12 major hospitals in 2000 were Methicillin resistant in Taiwan. In NICUs, MRSA were common nosocomial pathogens and accounted for >90% of *S. aureus* isolates since 1998. Standard infection control measures were implemented but were unable to control the spread of MRSA effectively. To identify infants with MRSA colonization and to implement cohort care for these infants, MRSA carriage was screened for infant admitted or transferred to NICUs.
Surajit Das et al., (2006) studied that the marine microorganism which are salt tolerant, provide an interesting alternative for therapeutic purposes. Marine microorganisms have a diverse range of enzymatic activity and are capable of catalyzing various biochemical reactions with novel enzymes. Halophilic microorganisms possess many hydrolytic enzymes and are capable of functioning under conditions that lead to precipitation of denaturation of most proteins. Further, it is believed that sea water, which is saline in nature and chemically closer to human blood plasma, could provide microbial products, in particular the enzymes, that could be safer having less toxicity or side effects when used for therapeutic applications to humans.

Summaiya Mulla et al., (2007) summarized that there was a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents. In Surat, South Gujarat, India for the period of 3 months from August - October 2004, processed the samples of pus, urine, blood, high vaginal swabs, sputum, throat swabs, drains and ear swabs received from New Civil Hospital, Surat. Among 135 Staphylococci 48 (35.55%) were coagulase positive. These coagulase positive Staphylococci isolates were screened for Methicillin resistance by a slide latex agglutination kit for the rapid detection of PBP2’ (Penicillin binding protein 2a). Sensitivity to Amikacin, Erythromycin, Clindamycin and Tetracycline were also carried out following Kirby Bauer disc diffusion method. Methicillin resistance among the *S. aureus* isolates was 39.5%. Resistance to all antibiotics tested among Methicillin resistance and sensitive, staphylococci was 26.3% and 6.8% respectively. Methicillin resistance was useful marker in selecting appropriate antimicrobial agents for treatment of *S. aureus* infections.

Van Loo et al., (2007) detected a novel chromogenic medium for MRSA, MRSA Select (Bio-Rad), with clinical samples in a public health laboratory in Netherlands. 3,000 samples were tested during January- March 2005, including 972 nose, 972 throat, 968 perineum, and 88 wound samples. Presumptive MRSA colonies appeared pink/mauve on the MRSA Select medium. The performance of MRSA Select medium was compared with the routine screening method. The colony morphology showed that isolates grew as pink/mauve colonies. The number of false-positive pink/mauve colonies increased after incubation from 20-48 hrs. The specificity decreased from 92% after 20 hrs incubation to 89% after 48 hrs incubation. In total 70 MRSA stains were isolated, 55 were detected by MRSA Select medium and 55 were detected by the routine screening
method. Sensitivity observed was 78.6% for both test procedures, and specificities were 99.5 and 100% respectively for the MRSA Select Medium and routine screening method.

Bera et al., (2007) revealed the prevalence of lysozyme resistance among staphylococci: that only pathogenic strains were resistant to lysozyme and muramic acid of the peptidoglycan of all resistant strains were O-acetylated, while all the non-pathogenic strains were sensitive and their muramic acid were not acetylated (Noreddine Benkerroum et al., 2008). Lysozyme resistance was considered as a virulence factor directly related to the expression of OatA gene.

Ocky Karna Radjsa et al., (2007) proved that the bioactive natural products from marine invertebrates have striking similarities to metabolites of their associated microorganisms including bacteria. The possible role of marine bacteria associated with sponges in providing solution to the problem of infection by pathogenic bacteria. Bacteria sponge associations that occur on the sponges could be great interest to search for potential use as new source of antibiotics in particular as a solution of the problem of supply of most bioactive compounds produced by reef’s invertebrates.

Sukarmi and Ocky Karna Radjasa, (2007) recently isolated compounds, mainly from sea cucumber, sea urchin and starfish with antitumour, antiviral, anticoagulant and antimicrobial activity. Hence the future of the echinoculture industry is closely linked to that of the fisheries. The most significant problem that has hampered the investigation of secondary metabolites in marine organism was low concentration. In marine invertebrates many highly active compounds contribute to <10^-6 % of the body wet weight. Due to limited amounts or limited quantity of the organism itself in amounts and nature of produced secondary metabolites. There has an increasing concerns regarding the discovery and development of pharmaceuticals since it has been perceived variously as sustaining and threatening conservation. There was an urgent need to take into account the bioethical consideration for sustainable use of reef’s invertebrates as the source of bioactive compounds.
According to Parungao et al., (2007) the WHO over prescription and improper use of antibiotics has led to the resistance of many pathogens. Clinically important bacteria, such as *S. aureus* are becoming resistant to commonly used antibiotics. New resistant strains emerge more quickly while the rate of discovery of new antibiotics is slowing down. Prakash et al., (2007) observed that emergence of antibiotic resistance in microorganism and their spread is threatening the medical community.

Venkateshwara Rao et al., (2008) verified sponges as one of the most productive marine ecosystems, with regard to presence of novel bio-active compounds. Few sponges (*n*=18) were collected from Palk bay and Gulf of Mannar waters of India and their methanol and dichloromethane (1:1) extracts were screened for larvicidal and insecticidal properties. Among them, around 40% of the test extracts were active against the fourth instar larvae of *Aedes aegypti* (Linn) and three to four day old of female houseflies, *Musca domestica* (Linn) at the concentrations of less than 100 ppm and 100 µg/insect respectively. Among the sponges *Psammaphysilla purpurea, Haliclona cribricutis, Dendrilla nigra, Haliclona pigmentifera* and *Petrosia testudinaria* could be used to obtain novel pesticidal molecules.

Asha Devi et al., (2008) isolated 91 bacterial colonies from water samples of five coastal regions of Southern India (Tamil Nadu). All these isolates were culture purified and screened for antimicrobial activity against a battery of human pathogens. Among these, 33% of the isolates (*n*=30) exhibited bacterial properties against pathogens. Morphological, cultural, physiological, biochemical, properties and molecular characterization, 16S rRNA sequencing and BLAST analysis indicated this strain as *Bacillus clausii* (100% similarity with GenBank sequences). The secondary metabolite (culture supernatant) produced by *B. clausii* MB9 at different salinities temperature and pH values was found to be stable at elevated temperature conditions. Moreover, the culture supernatant from the isolate remains active and retains its antimicrobial activities even the supernatant was subjected to 121°C. The results indicate that *B. clausii* MB9 produced a novel highly thermostable secondary metabolite showing a wide spectrum of antimicrobial activities.
Wijffels et al., (2008) initially wondered that out of 15 natural marine products, there are 45 marine derived natural products to be used as medical drugs in preclinical and clinical trials. To date, two of them have been developed into registered drugs. These are Prialt®, developed by Elon Pharmaceuticals from zinconotide, a product of a marine cone snail, and Yondelis®, developed by Pharmamar from trabectedin, a product from a tunicate. Whether bioactive compounds originate from sponges themselves, or from sponge associated microorganism or whether they could originate from the symbiotic relationship between sponges and microbes. The organisms which are responsible for production of the bioactive compounds have been demonstrated in only a limited number of cases and thus it is not appropriate to draw general conclusions. Bioactive compound shown to be produced by a sponge was stevensine from Axinella corruga which has complex chemical structure.

Boobathy et al., (2009b) studied the antibacterial activity of proteins from the marine sponge Sigmadocia fibulatus, collected from Tuticorin coast. Sponge with Chloroform and aqueous extracts yielded 6.8 gm and 5.5 gm from 500 gm of sponge respectively. Crude protein from the sponge were extracted, the concentration of 2.82 gm/mL in aqueous and 1.93 mg/mL in chloroform extract. The antimicrobial activity against bacterial pathogens showed clear inhibition zone against V. cholerae and A. niger in chloroform and aqueous extracts showed clear inhibition zone for Pseudomonas sp. and C. albicans. Both the extracts exhibited hemolytic activities which were estimated as 11.09 ht/mg of chloroform extract and 9.8 ht/mg of aqueous extract. On SDS-PAGE the crude protein yielded three well defined bands at 109.9, 28.2, 12.4 KDa in both the extracts. The Fatty acid profile showed the dominance of myristic acid (14.67%) in both extracts.

Muller, (2010) mentioned in the last decade the phylogenetically oldest metazoan phylum, in the Porifera (sponges) gained special interest. Mainly due to the introduction of molecular biological techniques solid evidence were elaborated which indicated that this phylum provides a cornucopia of new information which allows a grasping for the understanding of the dynamics of evolutionary processes. The species are rich sources for bio-prospecting, the translation of life science discoveries into
practical products for the benefit of the society. The field of bio-prospecting of Porifera may be of tremendous benefit for humans. Very promising is the potential application of bioactive compounds from Porifera in human therapy. In Japan arabino furanosyladenine (ara-A) drug is the most applied ointment against HSV. The number of sponge genes is higher than that in the ‘crown’ phyla allows also an explanation why the diversity of the secondary metabolites in sponges and other filter feeders was comparably so high. These animals in order to survive the environmental threads to form bioactive compounds from the secondary metabolites. The postoperative wound infections in S. aureus were common cause of nosocomial wound infections. Increased frequency of MRSA in hospitalized patients and possibility of Vancomycin resistance requires permanent control of MRSA spread. Wound swabs were examined from January 2006 to December 2008 and isolates were identified by conventional methods. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method as per NCCLS guidelines. A total of 5,755 wound swabs were examined: 938 (16.6%) and 4,817 (837%) were positive. S. aureus were isolated in 1,050 (220%) swabs and it was the most common cause of wound infections. MRSA were isolated from 124% samples in 2006, from 67% samples in 2007 and from 37% samples during 2008. Wound infections caused by MRSA dominated in the department of plastic surgery 244% and orthopedic surgery 241%. Antimicrobial susceptibility showed that 73% MRSA were sensitive to Vancomycin and Tetracycline (Sisirak et al., 2010).

The subject of this review is the biodiversity of marine sponges and associated microbes which have been reported to produce therapeutically important compounds. Production of a few identical compounds by entirely different host microbial associations has been detected in both terrestrial and marine environments. In the Demospongiae, microbial association is highly specific and so to the production of compounds. Though spatial and temporal variations are known to have a marked effect on the quality and quantity of bioactive compounds, only a few studies have covered these dimensions. The need to augment production of these compounds through tissue culture and mariculture has also been stressed (Tresa Remya et al., 2010).
Eliopoulos et al., (2011) suggested that the significant diversity in methicillin-resistant *Staphylococcus aureus* (MRSA) clones arising in the community worldwide, with considerable geographical differences in typical antimicrobial resistance profiles. The global epidemiology of community MRSA is remarkably heterogeneous. Several community clones of MRSA have a non-multidrug resistant antimicrobial profile, causing increased options for empirical and directed therapy of infections. The increasing non-β-lactam resistance in community clones of MRSA provides a timely warning for clinicians making decisions about therapy for patients potentially infected with these strains. The effective management of these infections were continued monitoring of global epidemiology and emerging drug resistance data which seems to be critical. Accurate drug resistance surveillance is crucial to recognize emerging resistance trends and to guide empirical antibiotic selection.

Chairman et al., (2012) tested the crude extracts from ethyl acetate and methanol for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Salmonella, Pseudomonas aeruginosa* and *Bacillus subtilis* from NCIM using disc diffusion method and MIC. Among the marine sponge 12 species screened were found to be more active than 2 sponges, *Spirastrella inconstans* var. moeandrina, and *Aurora globostellata* (Carter). It was observed that the ethyl acetate extracts of the marine sponge showed higher inhibitory activity for the selected bacterial species than methanol solvent extracts. The results revealed that the crude ethyl acetate extracts were effective in identifying pure antibacterial compound.