Review of Literature
3. REVIEW OF LITERATURE

Methicillin - Resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen world wide. It is still one of the major problems of drug resistance and it should be a frequent and an important human pathogen both in community and in hospital. Methicillin resistant *Staphylococcus aureus* (MRSA) is defined by the production of a specific penicillin binding proteins and resistance is regulated by *mecA* gene.

Methicillin was first introduced in 1959 to treat infections caused by Penicillin resistant *Staphylococci*. In 1961, there are reports of artificial induction of Methicillin resistance in *Staphylococci* and by 1963 appeared the first infections with Methicillin resistant *Staphylococcus aureus*. The first three infections were reported by Harding in 1963 in United Kingdom but soon these infections were documented from other European countries, Japan, Australia and the USA (1968).

3.1. Emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin-Resistant *Staphylococcus aureus* was first reported in Britain in 1960 and it increases infection was seen in western countries in the 1970s. In 1997-98, the increased incident of EMRSA was reported in all over 1,200 increased incidents. MRSA infection in intensive care unit and normal new born nurseries in Japan were investigated and various methods of prevalent transmissions were evaluated (Andrew E. Simor, et al., 2002).

Durmaz, et al., (1997) have concluded that the prevalence of Turkish isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Nosocomial and community infections and their antibiotic resistant patterns. A total of 383 Staphylococcus aureus strains were identified from different patients. The prevalence of methicillin resistance among *Staphylococcus aureus* strains was 31.3% (120/383). The proportions of MRSA isolated from Nosocomial and community infections were 26.4% (46/174) and 35.4% (74/209), respectively.
Nuno A. Faria, et al., (2005) have focused that the strict infection control measures introduced during the 1970s have kept the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections extremely low in Denmark. Nevertheless, similarly to other countries, MRSA infections began to appear in the community in the late 1990s. A nationwide surveillance program has collected and stored all MRSA isolates since 1988 and, since 1999, clinical information, detailed epidemiological and molecular analysis of the 81 MRSA infections identified in Denmark in 2001.

Pierre-Yves Donnio, et al., (2002) have reported that the distributions of the antibiotic patterns in a population of *Staphylococcus aureus* isolates from a teaching hospital were studied over a 9-years period. The results indicate that the existence of successive major epidemic methicillin-resistant strains and the emergence of a Methicillin-susceptible strain with an unusual resistance pattern. The findings suggest that this Methicillin-susceptible *Staphylococcus aureus* strain could be derived from the dominant Gentamycin-susceptible Methicillin-Resistant *Staphylococcus aureus* strain with the loss of a 40-kb DNA fragment.

Trindade, et al., (2003) have reported that *Staphylococcus aureus* has long been recognized as an important pathogen in human disease. Serious *Staphylococcal* infections can frequently occur in inpatients and may lead to dire consequences, especially as to therapy with antimicrobial agents.

Paul D. Brown and Charles Ngeno (2007) have been reported that the antimicrobial susceptibility patterns and prevalence of methicillin resistance among *Staphylococcus aureus* isolates from hospital and community sources in Southern Jamaica. Eighty isolates of *Staphylococcus aureus* obtained from hospital and community-based patients with *Staphylococcal* infections were collected. The overall prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was 23%. This is the first report of MRSA from this region of Jamaica. Because methicillin resistance is associated with multiple-drug resistance in *Staphylococcus aureus*, it is imperative that surveillance
initiatives be focused on the hospital and community in order to monitor and limit the spread of this organism.

Multidrug - Resistant bacteria, such as Methicillin - Resistant Staphylococcus aureus (MRSA), are endemic in health care settings in United States and other many countries of the world. Colonization with Staphylococcus aureus or MRSA is relatively common in both healthy and hospitalized individuals; most often involves the anterior nares, and is frequently asymptomatic. Patient-to-Patient transmission of MRSA within health care settings primarily occurs via carriage on the hands of healthcare workers. The Society for Healthcare Epidemiology of America (SHEA) has developed guidelines for the prevention of transmission of MRSA within health care settings, and chief among the recommendations is an emphasis on adherence to hand hygiene guidelines have been investigated by David K. Henderson (2006).

3.2. Mechanism of Methicillin Resistance

Globalization has entailed a massive increase in trade and human mobility facilitating the rapid spread of infectious agents, including those that are drug resistant. Staphylococcus aureus is a frequent and an important human pathogen both in the community and in hospitals.

In 1992, Kloos, et al., have demonstrated that the Staphylococcus aureus can acquire resistance through extra chromosomal plasmids, through additional genetic information delivered by transposons, and through mutations in chromosomal genes. Resistance is achieved through a variety of mechanisms, including enzymatic inactivation of the drug (as with Penicillinase, which cleaves the β-lactam ring of penicillins), alteration of the drug target to prevent binding, and enhanced removal of the drug from the host tissue.

A particularly serious threat to human health is posed by Methicillin-resistant Staphylococcal strains which have acquired molecular mechanisms
to evade the action of β-lactam antibiotics (BLAs). Full expression of high-level methicillin resistance involves a complex network of molecules and depends primarily on sufficient expression of a penicillin-binding protein with low sensitivity towards BLAs. Other factors include the fine-tuned regulation of autolytic activity of cell-wall components, as well as an optimal rate of peptidoglycan precursor formation and a highly specific peptidoglycan precursor structure (Goretti Mallorqui-Fernandez, et al., 2004).

All clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates examined so far contain the meca gene, a 2130bp stretch of DNA of non-staphylococcal origin which, together with a larger block (up to 40-60 kb) of foreign DNA, is incorporated into the Staphylococcal chromosome. meca encodes for the 78kb penicillin-binding protein (PBP) 2A, which has low affinity for β-lactam antibiotics have been concluded by Herminia de Lencastre, et al., (1994). Vijay K. Sharma, et al., (1998) have been reported that the Methicillin resistance in *Staphylococci* is mediated by PBP2a, a penicillin binding protein with low affinity for β-lactam antibiotics. The gene encoding PBP2a, meca, is transcriptionally regulated in some clinical isolates by mecr1 and mecl, genes divergently transcribed from meca that encode a signal transducer and repressor, respectively.

Yuki Katayama, et al., (2005) have focused the *Staphylococcal* methicillin resistance determinant, meca, resides on a mobile genetic element, *Staphylococcus* chromosome cassette mec (SCCmec). The distribution of SCCmec in nature is limited to relatively few clonal complexes of related Methicillin-resistant *Staphylococcus aureus* (MRSA). We investigate the potential role of the host chromosome in the transformability and expression of meca in 103 naturally occurring Methicillin-susceptible *Staphylococcus aureus* clinical isolates. The isolates, which had been genotyped previously by multilocus sequence typing, were classified into one of two mutually exclusive categories based on whether the isolates belonged to “major” MRSA lineages or to “other” lineages that are never or occasionally MRSA. These data support the hypothesis that the presence of meca within relatively few clonal
complexes is partly due to genetic factors that are permissive of \textit{mecA} and its gene product.

Full characterization of Methicillin - Resistant \textit{Staphylococcus aureus} (MRSA) requires definition of not only the bacterial genetic background but also the structure of the complex and heterologous \textit{mec} element these bacteria carry, which is associated with drug resistance determinant \textit{mecA}. The development, validation and application of a multiplex PCR strategy that allows quick presumptive characterization of the \textit{mec} element types based on the structural features that were shown to be typical of \textit{mec} elements carried by several MRSA clones. The strategy was validated by using a representative collection of pandemic MRSA clones in which the full structure of the associated \textit{mec} elements was previously determined by hybridization and PCR screenings and also by DNA sequencing (Duarte C. Oliveira and Herminia de Lencastre, 2002).

Cotter, et al., (1997) have investigated that the continuing increase in numbers of isolates is reported from Irish hospitals each year. 48 MRSA strains were isolated in the Cork University Hospital were analyzed between January and July 1995 using a one-tube triplex-polymerase chain reaction, where in three genes, the methicillin resistance gene (\textit{mecA}), \textit{femA} and extra cellular thermo nuclease gene, \textit{nuc}, were simultaneously amplified. Methicillin-Sensitive \textit{Staphylococcus aureus} (MSSA) and Coagulase-negative \textit{Staphylococci} (CNS) were also tested and the assay was found to be MRSA specific.

3.3. MRSA in Children

MRSA was found to be highly prevalent in patients in the Neonatal Intensive Care Units (NICU). Recently, a new neonatal exanthematous disease called neonatal toxic shock syndrome like exanthematous disease (NTED) caused by toxic shock Syndrome toxin-1 (TSST-1) produced from MRSA, has developed in Japan (Takahashi, \textit{et al.}, 1998).
Nosocomial Methicillin-Resistant *Staphylococcus aureus* (MRSA) infection in infants has become a serious concern and a new means of preventing the transmission of MRSA in the community needs to be considered. The nasal mupirocin treatment on 10 infants who were MRSA-positive either in the nose or the pharynx and evaluated the effect of mupirocin on the eradication of MRSA. Eradication of MRSA from the nose was successful in two cases and eradication from the pharynx in six (66.6%) of nine cases. The number of treatments required to achieve eradication varied; within three courses for nose carriers and from one to seven courses for pharynx carriers. Eradication was unsuccessful even after five to seven treatments in three pharynx-limited carriers. These data suggest that the effect of nasal mupirocin treatment on pharynx-colonized MRSA is limited and that repetitive treatment is necessary in some cases have been reported by Takahiro Hayakawa, *et al.*, (2000).

Joel Guss and Ken Kazahaya (2007) have concluded that the determination of the microbiology, particularly the prevalence of MRSA, in pediatric patients with community-acquired bacterial lymphadenitis and the culture results of patients under the age of 18 who underwent trans-cervical surgical drainage of abscessed lymph nodes between the years 2000 and 2006. Sixty-two patients were identified for whom microbiology data were available. The most common organism was *Staphylococcus aureus* (63% of positive cultures); followed by β-hemolytic group A *Streptococcus* (22%). Of *Staphylococcus aureus* isolates, 27% were Oxacillin-resistant (MRSA). All Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates were sensitive to clindamycin and Trimethoprim/Sulfamethoxazole; 63% were sensitive to ciprofloxacin, and 25% sensitive to erythromycin. Resistance to clindamycin, a drug commonly used to treat MRSA, is prevalent amongst the Methicillin-Sensitive *Staphylococcus aureus* (MSSA). This has important implications regarding the empiric treatment of lymphadenitis in children.

Orbital complications of sinusitis (OCS) are uncommon in children and are extremely rare in the neonatal period and the related literature also
inadequate. Reported surgical techniques to manage orbital abscess collection involve either an external ethmoidectomy or transconjunctival approach for decompression and drainage. The case of a 13 day-old male who was managed with endoscopic drainage of a Methicillin-resistant *Staphylococcus aureus* (MRSA) orbital abscess have been reported by Aaron Rogers, *et al.*, (2007).

### 3.4. MRSA Infections

Infections caused by MRSA in compromised hosts pose a serious problem all over the world, because MRSA strains are resistant to numerous antibiotics and can be transmitted from patient to patient via transiently colonized hands of hospital personnel (Brumfit and Hamilton-Miller, (1989) and Mulligan, *et al.*, (1993).

Kunio Takeuchi, *et al.*, (2001) have shown that the clinical features of Methicillin-Resistant *Staphylococcus aureus* (MRSA) enteritis in the surgical ward. The underlying diseases were included as gastric cancer, colorectal cancer, recurrent cancer and bowel obstruction following gastrectomy. In 13 cases MRSA enteritis developed within 6 days of operation. 10 strains of MRSA were isolated from stools, 8 from gastric juice, and 3 from intra-abdominal exudates. 10 patients were treated with Vancomycin given through a nasogastric tube and 2 through a nasogastric tube and by drip intravenous infusion. 15 patients survived and 2 patients were died.

Guilberme Santoro-Lopes, *et al.*, (2005) have focused that the Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a frequent cause of infection after orthotropic liver transplantation (OLT). Colonization with MRSA is associated with a higher risk of infection. The risk of colonization with MRSA after OLT is still unclear. Surveillance cultures of nasal swab specimens were performed within the 1st 72 hours of hospital admission and subsequently, on weeks 2, 6, 13, and 26. Patients whose baseline cultures revealed nasal carriage of MRSA were excluded. In conclusion, nasal carriage of MRSA is frequently acquired after OLT. Periodic postoperative screening for
MRSA carriage should be an integral component in programs designed to reduce nosocomial MRSA transmission in these patients.

Pesavento, et al., (2007) have reported that the Methicillin - Resistant Staphylococcus aureus (MRSA) has emerged as a risk factor for patients in general population and particularly in immuno compromised patients. As a matter of fact, it can produce serious infections that may then evolve in septicemia. However, transmission of MRSA from food to people can represent a serious problem only for immuno compromised people. 42 strains of Staphylococcus aureus were isolated from 176 samples of raw meat (poultry, pork and beef) during a one-year survey. Each strain was tested against 12 antimicrobial to verify antibiotic resistance. We found no evidence of methicillin, teicoplanin or Vancomycin-resistance, but a lot of multi-resistant microorganisms, i.e. resistant to three or more antibiotics. The result confirms the hypothesis that antibiotics resistance is present not only in nosocomial bacteria, but also in community environments microorganisms.

3.5. Microbiological & Molecular Diagnosis

Lipovitellin-Salt-Mannitol (LSM) plate medium was examined for its ability to directly isolate, recover, and presumptively identify Staphylococcus aureus from 418 clinical specimens. For 298 specimens used for screening, LSM agar medium was compared with the other conventional media used, Mannitol salt agar (MSA), 5% horse blood agar (HBA), and Phenolphthalein phosphate agar (PPA), to detect and recover Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus. LSM agar may be an alternative plate medium for large hospitals that perform extensive screening for the detection and isolation of Staphylococcus aureus have been identified by John Merlino, et al., (1996).

John Merlino, et al., (2000) have identified that the new chromogenic plate medium, CHROMagar Staph aureus (CHROMagar, Paris, France), for the identification of Staphylococcus aureus on the basis of colony pigmentation. They compared the abilities of CHROMagar Staph aureus, thermostable
nuclease (DNase), and Mannitol salt agar (MSA) to identify *Staphylococcus aureus* isolates and discriminate between *Staphylococcus aureus* and Coagulase - Negative *Staphylococci* (CNS). CHROMagar *Staph aureus* proved to be more sensitive and specific than DNase and MSA, allowing a reliable, simple, and rapid method for the identification of *Staphylococcus aureus* isolates.

Heiman Wertheim, *et al.*, (2001) have reported that the phenyl Mannitol broth containing ceftizoxime and aztreonam for the detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) with reference MRSA strains and with clinical samples (*n*=1,098). All reference MRSA strains induced colour change in PHMB after 24 to 72 hours of incubation. 40 MRSA strains were detected with PHMB, compared with only 23 detected with routine method. This selective broth significantly improved the rate of MRSA detection.

Flayhart, *et al.*, (2005) have identified that the total of 1133 nasal samples were plated to Sheep blood agar and BBL\textsuperscript{TM}CHROMagar\textsuperscript{TM}MRSA (C-MRSA). MRSA appeared as mauve colonies on C-MRSA and all other organisms including Methicillin susceptible are inhibited or produced a distinctly different colony colour.

Derek F.J. Brown, *et al.*, (2005) have concluded that the detection of MRSA in screening samples and the detection of reduced susceptibility to glycopeptides in *Staphylococcus aureus*. There are currently several developments in screening media and molecular methods included as tube and Slide Coagulase test, Latex agglutination test, DNase test, Heat stable nuclease test, Dilution methods, E-test method, Agar Screening test, Quenching fluorescence test, PCR analysis and Southern blotting.

Two new selective media, Oxacillin resistance screening agar base (ORSAB) and CHROMagar *Staphylococcus aureus* (CSA) were evaluated for the identification of *Staphylococcus aureus* and for screening of Methicillin resistance by the addition of antimicrobial agents to these media. For the identification of *Staphylococcus aureus*, both media performed better after 24
hrs than after 48 hrs of incubation. For screening of Methicillin resistance, antibiotic supplements were added to both media. The sensitivity was lower after 24 hrs (CSA, 58.6%; ORSAB, 84.2%) and increased significantly after 48 hrs (CSA, 77.5%; ORSAB, 91.4%). At both time intervals ORSAB was significantly more sensitive than CSA. Finally, Jan Kluytmans, et al., (2002) have concluded that the screening of MRSA, ORSAB performs better than CSA medium.

In *Staphylococcus aureus*, meCA and femA are the genetic determinants of methicillin resistant. By using a multiplex PCR strategy, 310- and 686-bp regions of the meCA and femA genes, respectively, were amplified to identify susceptible (lacking meCA) and resistant (meCA+) *Staphylococci* and to differentiate *Staphylococcus aureus* (femA+) from Coagulase-negative *Staphylococci* (lacking femA). One hundred sixty-five Staphylococcal strains were tested. All 72 methicillin-resistant Strains were found to be meCA+, and 92 of the 93 susceptible isolates lacked meCA. The possibility of directly detecting the meCA and femA genes in blood samples was also investigated. This technique, which can be successfully performed with blood sample, could be a useful tool in the diagnosis and treatment monitoring of *Staphylococcal* infections reported by Pascal Vannuffel, et al., (1995).

Lan Mo and Qi-nan Wang (1997) have concluded the comparison of polymerase chain reaction (PCR), Southern blot, and routine susceptibility testing for determination of methicillin resistance (to detect the meCA gene) in clinical isolates of *Staphylococci*. The presence or absence of a methicillin-resistant gene (meCA) in 228 clinical isolates of *Staphylococci* was examined by PCR and Southern blot analyses. A total of 57 of 58 Oxacillin-resistant *Staphylococcus aureus* strains were meCA-positive, whereas 3 of 126 Oxacillin-susceptible strains were meCA-positive. For 21 Oxacillin-resistant Coagulase-negative *Staphylococci*, 100% of the strains were meCA-positive, but 9 of 23 Oxacillin-Susceptible Coagulase-Negative *Staphylococci* were meCA-positive. The PCR test identified Methicillin-Resistant *Staphylococci* in less than 3 hours, using as few as 300 cells or 3 pg crude extract of DNA as the PCR
template. The results of the PCR test correlated well with those of DNA hybridization. Dot blot hybridization could detect as little as 4 ng DNA. Identification of Methicillin-Resistant Staphylococci by PCR (Confirmed by DNA hybridization) offers a specific, sensitive, and serves as a guide for the rational treatment of infections caused by Staphylococci.

Richard J. Jaffe, et al., (2000) have reported that Methicillin-Resistant Staphylococci (MRS) are one of the most common causes of nosocomial infections and bacteraemia. The use of the PCR is a rapid and simple process for the amplification of target DNA sequences, which can be used to identify and test bacteria for antimicrobial resistant.

Manisha Mehrotra, et al., (2000) have identified the multiplex PCR assay for detection of genes for Staphylococcal Enterotoxins A to E (entA, entB, entC, entD, and entE), toxic shock syndrome toxin 1 (tst), Exfoliative toxins A and B (etaA and etaB), and intrinsic methicillin resistance (mecA) was developed. Detection of femA was used as an internal positive control. The multiplex PCR assay combined the primers for sea to see and femA in one set and those for eta, etb, tst, mecA, and femA in the other set. Validation of the assay was performed using 176 human isolates of Staphylococcus aureus. The assay offers a very specific, quick, reliable, and inexpensive alternative to conventional PCR assays used in clinical laboratories to identify various Staphylococcal toxin genes.

Louie, et al., (2000) have reported the probe-based Velogene Rapid MRSA Identification Assay (ID Biomedical Corp., Vancouver, British Columbia, Canada) and the latex agglutination MRSA-screen (Denka Seiken Co., Tokyo, Japan) were evaluated for their ability to identify Methicillin-resistant Staphylococcus aureus (MRSA) and to distinguish strains of MRSA from borderline Oxacillin-Resistant Staphylococcus aureus (BORSA; mecA-negative, Oxacillin MICs of 2 to 8 µg/ml). The Velogene is a 90-min assay using a chimeric probe to detect mecA gene. MRSA-Screen is a 15-min latex agglutination test with penicillin-binding protein 2a antibody-sensitized latex
particles. The two methods were compared with BBL Crystal MRSA ID system (Becton Dickinson, Cockeysville, Md.) and with PCR for mecA gene detection. All assays performed well for the identification of MRSA with sensitivities and specificities for Velogene, MRSA-Screen, and BBL Crystal MRSA ID of 98.5% and 100%, 98.5% and 100%, and 98.5% and 98%, respectively.

William J. Mason, et al., (2001) have concluded that the development of a multiplex PCR protocol for the diagnosis of Staphylococcal infection. The protocol was designed to (i) detect any Staphylococcal species to the exclusion of their bacterial pathogens (based on primers corresponding to Staphylococcus - specific regions of the 16S rRNA genes), (ii) distinguish between Staphylococcus aureus and the Coagulase-Negative Staphylococci (CNS) (based on amplification of the Staphylococcus aureus-specific clfA gene), and (iii) provide an indication of the likelihood that the Staphylococci present in the specimen are resistant to Oxacillin (based on amplification of the mecA gene).

George Sakoulas, et al., (2001) have reported that the Methicillin-Resistant Staphylococcus aureus (MRSA) is responsible for an increasing number of serious nosocomial and community - acquired infections. Phenotypic heterogeneous drug resistance (hetero resistance) to Anti-Staphylococcal beta-lactams affects the results of susceptibility testing. This findings have compared the MRSA-Screen latex agglutination test (Denka Seiken Co., Ltd., Tokyo, Japan) for detection of PBP2a with agar dilution, the VITEK-1 and VITEK-2 systems (bilMerieux, St.Louis, Mo.) and the Oxacillin agar screen test for detection of MRSA, with PCR for the mecA gene used as the “Gold Standard” assay.

Perez-Roth, et al., (2001) have concluded that the multiplex PCR assay for the detection of clinically relevant antibiotic resistant 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 resistant), ileS-2 (encoding high-level mupirocin resistant), and \textit{femB} (encoding a factor essential for methicillin resistant) genes, respectively, from a single colony in a single reaction tube. The \textit{femB} PCR fragment allows the specific identification of \textit{Staphylococcus aureus}. Validation of the method was performed using 50 human isolates of Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and the appropriate control strains.

Galdiero, \textit{et al.}, (2003) have focused about two hundred and twenty strains of \textit{Staphylococci} isolated in Naples, Italy, were surveyed for the distribution of the \textit{mecA}, the structural gene for penicillin-binding protein 2a, which is the genetic determinant for Methicillin-Resistance in \textit{Staphylococci}. Screening by a cloned \textit{mecA}, revealed that of 220 strains, 43 were Methicillin-Resistant (19.5\%) and 177 were Methicillin-Susceptible (80.5\%). Among the 43 resistant strains 23 (53.5\%) carried \textit{mecA} in their genome and 20 (46.5\%) did not carry \textit{mecA}, in spite of their resistance to methicillin. A quantitative analysis of the patterns divided strains into four different clusters for Methicillin-Resistant \textit{mecA}-negative and two different clusters for Methicillin-Resistant \textit{mecA}-positive \textit{Staphylococcal} isolates.

Birgit Strommenger, \textit{et al.}, (2003) have reported that the multiplex PCR assay for the detection of nine clinically relevant antibiotic resistance genes of \textit{Staphylococcus aureus}. Conditions were optimized to amplify fragments of \textit{mecA} (encoding Methicillin resistance). The multiplex PCR assay was evaluated on 30 different \textit{Staphylococcus aureus} isolates, and the PCR results correlated with the phenotypic antibiotic resistance data obtained by the broth micro dilution assay. The multiplex PCR assay offers a rapid, simple, and accurate identification of antibiotic resistance profiles and could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic resistance determinants in epidemiological studies.

Patrice Francois, \textit{et al.}, (2003) have identified the rapid procedure for the detection and identification of Methicillin-Resistant \textit{Staphylococcus aureus}
(MRSA) directly from sterile sites or mixed flora samples. After a rapid conditioning of samples, the method consists of two main steps: (i) immuno-magnetic enrichment in *Staphylococcus aureus* and (ii) amplification-detection profile on DNA extracts using multiplex quantitative PCR. The triplex qPCR assay measures simultaneously the following targets: (i) *meca* gene, conferring methicillin resistance, common to both *Staphylococcus aureus* and *Staphylococcus epidermidis*; (ii) *femA* gene from *Staphylococcus aureus*; and (iii) *femA* gene from *Staphylococcus epidermidis*. This 96-well format assay allowed analysis of 30 swab sample per run and detection of the presence of MRSA with exquisite sensitivity compared to optimal culture-based techniques. The complete protocol may provide results in less than 6 hours, thus allowing prompt and cost-effective implementation of contact precautions.

Aziz Japoni, *et al.*, (2004) have concluded that the Nosocomial infection caused by Methicillin-resistant *Staphylococci* poses a serious problem in many countries. The aim of this study was to rapidly and reliably detect Methicillin - Resistant *Staphylococci* in order to suggest appropriate therapy. The presence or absence of the Methicillin-resistant gene in 115 clinical isolates of *Staphylococcus aureus* and 50 isolates of Coagulase-Negative *Staphylococci* (CNS) was examined by PCR.

Bo Shopsin, *et al.*, (2004) have focused that the recent reports indicate that community-acquired Methicillin - Resistant *Staphylococcus aureus* (MRSA) infection are increasing and may now involve persons without risk factors predisposing for acquisition. To estimate the extends of community MRSA in New York City, the prevalence of *Staphylococcus aureus* and MRSA nasal colonization in a well-patient population of 500 children and 28% for guardians. Repetitive DNA Sequence PCR (rep-PCR) may be used for screening due to its practicality, low cost and reproducibility. Because of its high discriminatory power Pulsed-Field gel Electrophoresis (PFGE) still remains the gold standard for MRSA typing.
Aziz Japoni, et al., (2004) have reported that the DNA extraction procedure for PCR performance by omission of acromopeptadiase and Proteinase K digestion, phenol/chloroform extraction and ethanol precipitation. All isolates with MIC>8 µg/ml showed positive PCR. No difference in PCR detection has been observed when normal and modified DNA extractions have been performed. Our modified DNA extraction can quickly detect Methicillin - Resistant *Staphylococci* by PCR. The advantage of rapid DNA extraction extends to both reduction of time and cost of PCR performed.

The utility of real-time polymerase chain reaction (RT-PCR) testing for detection of Methicillin - Resistant *Staphylococcus aureus* (MRSA) directly from positive blood culture bottles was evaluated. The total of 142 blood culture showing gram-positive cocci in clusters was tested for MRSA by PCR analysis (SmartCycler) via detection of *mecA* and *orfX* genes. PCR analysis directly from the blood culture bottle required a total time of 120 min (45 min for preparation and 75 min for the reaction). By comparison, conventional laboratory procedures required between 48 and 72 hrs. The overall test accuracy was 97% with a high positive likelihood ratio and a low negative likelihood ratio John Stratidis, et al., (2007).

The minimal inhibitory concentrations of penicillin against 96 strains of group B *Streptococci* and of Methicillin against 10 strains of *Staphylococcus aureus* were unrelated to the growth phase of test bacteria (Kwang Sik Kim and Bascom F. Anthony, 1981).

β-Lactamase testing of clinical isolates of *Staphylococci* may be performed in commercial broth micro dilution minimum inhibitory concentration plates, using a chromogenic cephalosporin reagent directly in a well containing a non-inhibitory concentration of a semisynthetic penicillin which serves as a inducer and a total of 115 *Staphylococcal* isolates tested in 0.25 to 0.50 µg of methicillin per ml in Mueller-Hinton broth showed 100%
correlation with β-Lactamase tests performed on Mueller-Hinton agar, using 1μg Oxacillin disk as an inducer (Kimberly A. Horton, et al., 1982).

Minimal bactericidal concentrations (MBCs) of nine antimicrobial agents were determined for clinical isolates by a replica plating method. Membranes were placed on the antibiotic-containing plates and the organisms replicated onto the membranes. After 18 hrs of incubation, the minimal inhibitory concentrations (MICs) were determined, the membranes were transferred to antibiotic-free plates and incubated a further 18 hrs and the MBCs determined. MICs and MBCs were also determined in broth. The reproducibility of the 'membrane' method and the agreement of these results for MIC and MBC with the agar and/or broth methods were satisfactory for most antibiotics, within one two-fold dilution (Clarence J. Fernandes, et al., 1985).

Minimum Inhibitory Concentrations were determined for selected antimicrobial agents against 872 bacteria isolated from intramammary infections in heifers in New Zealand and Denmark. These values were reported in micrograms per milliliters. Antimicrobial agents tested against isolates from New Zealand were Penicillin, Cloxacillin, Cephapirin, Ceftiofur, Novobiocin, ofloxacin, Erythromycin, and Pirlimycin. The minimum inhibitory concentration values that inhibit 90% of the strains tested against the *Staphylococcus* spp. ranged from 0.5 to 1.0 for all antimicrobials and the values for *Staphylococci* from New Zealand and Denmark were similar to values reported for US isolates (Salmon, et al., 1998).

### 3.6. Treatment by Antibiotics

In vitro activity of Seven Anti-*Staphylococcal* antibiotics alone and in combination with four Aminoglycoside antibiotics against 35 clinical isolates of *Staphylococcus aureus* from blood cultures of patients with endocarditis or septicemia were studied. The combination of nafcillin - Gentamycin or nafcillin - tobramycin when compared with nafcillin alone killed significantly more *Staphylococcus aureus* at 6 hrs for 33 of 35 isolates. A significant
decrease in viable colony-forming units at 24 and 48 h was demonstrated for a smaller number of isolates (Chatrchai Watanakunakorn and Cheryl Glotzbecker, 1974).

Enoxacin is a new quinolone carboxylic acid compound and its activity against 740 bacterial isolates including methicillin sensitive and Methicillin-Resistant *Staphylococcus aureus* isolates. Although most of the *Staphylococcus aureus* were inhibited by 3.1μg/ml. There was no major difference between minimal inhibitory concentrations and minimal bactericidal concentrations. Resistance frequency development was <10⁻⁹ for most bacterial species (Nai-Xun Chin and Harold C. Neu, 1983).

Tigecycline and Daptomycin are the potent antibacterial compound in advanced stages of clinical trails. These novel agents target multiply resistant pathogenic bacteria. Daptomycin is principally active against Gram-positive bacteria, while Tigecycline has broad-spectrum activity. Tigecycline was more active against *Staphylococcal, Enterococcal* and *Streptococcal* pathogens. The activity of daptomycin equaled that of Tigecycline against the Glycopeptide-intermediate *Staphylococcus aureus* (GISA) only when the test medium was supplemented with excess calcium (75mg/liter). These data suggest that Tigecycline and daptomycin may offer therapeutic options against clinically relevant resistant pathogens for which current alternatives for treatment are limited (Peter J. Petersen, *et al.*, 2002).

*Staphylococcal* infections are a common and significant clinical problem in medical practice. Most strains of *Staphylococcus aureus* are now resistant to penicillin, and Methicillin-Resistant strains of *Staphylococcus aureus* (MRSA) are common in hospitals and are emerging in the community. All serious MRSA infections should be treated with parenteral Vancomycin or, if the patient is Vancomycin allergic, teicoplanin. Nosocomial strains of MRSA are typically multi-resistant (mrMRSA), and mrMRSA strains must always be treated with a combination of two oral antimicrobials, typically rifampicin and
fusidic acid because resistance developed rapidly if they are used as single agents (Rayner, C., and W.J. Munckhof, 2005).

The PPI-0903M is a novel N-phosphono-type Cephalosporin active against Oxacillin-resistant *Staphylococci* and many other gram-positive organisms and evaluated the in vitro activity and spectrum of PPI-0903M against 1,478 recent clinical isolates collected from 80 medical centers. PPI-0903M demonstrated broader in vitro activity against gram-positive bacteria, particularly against Multidrug-resistant *Staphylococci* and *Streptococci* of current clinical concern (Helio S. Sader, *et al.*, 2005).

A total of 345 Coagulase positive *Staphylococci* and 187 MRSA were isolated and identified the incidence of MRSA in intensive care units and burn centre was 23.4% and 29.6%, respectively. The resistant rate of MRSA were 29.9% for Trimethoprim - Sulfamethoxazole, 60.8% for Clindamycin, 71.8% for erythromycin, 7.7% for teikoplanin, 90.1% for gentamycin, 88.8% Ofloxacin, 88.1% for Norfloxacin and 100% for penicillin. All isolates were found to be sensitive against Vancomycin. Finally, Lutuf Savas, *et al.*, (2005) have identified that the Vancomycin as an effective antimicrobial agent against multi-drug resistant MRSA infections.

Kalsoom Farzana and Abdul Hameed (2006) have proposed that the resistance pattern of clinical isolates of *Staphylococcus aureus* against five groups of antibiotics includes Penicillin group, Cephalosporin group, Aminoglycoside group, Quinolone group and other antibiotics. Antibiotic strains were more prevalent in pus samples than the other clinical isolates (Blood and Urine). The selected strains of *Staphylococcus aureus* showed the resistance rate against Ampicillin (92%), Cephradine (60%) and Gentamycin (58%) and intermediate resistance was found in the case of Vancomycin (38%), in hospitalized and non-hospitalized patients.

The discovery, isolation, chemical and biological characterization of a new antibiotic compound, 7-0-Malonyl Macrolactin A (MMA), produced by a *Bacillus subtilis* soil isolate. MMA is a bacteriostatic antibiotic that inhibits a
number of Multidrug-Resistant gram-positive bacterial pathogens, including Methicillin - Resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococci (VRE), and a small-colony variant of Burkholderia cepacia (Magally Romero-Tabarez, et al., 2006).

The *in vitro* activities of 22 antimicrobial agents, including ceftobiprole, daptomycin, and Tigecycline, against 511 Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from 112 Belgian hospitals were studied by using the CLSI agar dilution method. The isolates were characterized by pulsed-field gel electrophoresis (PFGE) analysis and by PCR detection of determinants of resistance to Aminoglycosides, Macrolides-Lincosamides-Streptogramins, and tetracyclines. These data indicate excellent activity of the newly developed agents ceftobiprole, daptomycin, and Tigecycline against MRSA isolates recently recovered from hospitalized patients in Belgium, supporting their therapeutic potential for nosocomial MRSA infections (Olivier Denis, et al., 2006).

The synergistic effects of daptomycin plus Gentamicin or Rifampin were tested against 50 *Staphylococcus aureus* strains, with daptomycin MICs ranging between 0.25 and 8μg/ml. Daptomycin sub-MICs combined with Gentamicin concentrations lower than the MIC yielded synergy in 34 (68%) of the 50 strains. Daptomycin combined with Rifampin yielded synergy in one vancomycin-intermediate *Staphylococcus aureus* strain only, and virtually all synergy occurred between daptomycin and Gentamicin (Kim Credit, et al., 2007).

Daptomycin is a cyclic lipopeptide with potent activity and broad spectrum against Gram-positive bacteria currently used for the treatment of complicated skin and skin structure infections and bacteraemia, including right sided endocarditis. A total of 4,640 strains from 23 medical centers located in 10 European countries were tested for susceptibility by reference broth micro dilution methods. Mueller-Hinton broth was supplemented to 50mg/L Ca++ for testing Daptomycin. All *Staphylococcus aureus* strains were
inhibited at Daptomycin MIC of 1 mg/L (MIC<sub>50/90</sub>, 0.25/0.5 mg/L; 100.0% susceptible) and only one Coagulase-negative <i>Staphylococci</i> strain (0.1%) showed an elevated (>1 mg/L) Daptomycin MIC value (4 mg/L). Based on these results, Daptomycin appears to be an excellent therapeutic option for serious infections caused by Oxacillin-Resistant <i>Staphylococci</i> (Helio S Sader, <i>et al.</i>, 2007).

Treatment of infections caused by resistant bacterial pathogens mainly relies on two therapeutic modalities: development of new antimicrobials and use of combinations of available antibiotics. Double (Vancomycin or Teicoplanin with either Levofloxacin or Cefotaxime) and triple (Vancomycin or Teicoplanin + Levofloxacin + one among Amikacin, Ceftazidime, Cefepime, Imipenem, Piperacillin/Tazobactum) combinations were evaluated by means of checkerboard assay and time kill curves. Mutational rates of single and combined drugs at antimicrobial concentrations equal to the resistance breakpoints were also calculated. In vitro evidence of synergy between glycopeptides, Fluoroquinolones (Levofloxacin) and β-lactams and of reduction of mutational frequencies by combinations are suggestive for a potential role in empirical therapy of severe pneumonia with suspected MRSA etiology (Lorenzo Drago, <i>et al.</i>, 2007).

3.7. Treatment by Chemical Agents

Seckin Ozden, <i>et al.</i>, (2004) have reported that the series of 4 - (5, 6 - dichloro - 1H - benzimidazol - 2 - yl) - N - substituted Benzamides were synthesized and evaluated for antibacterial and antifungal activities against <i>Staphylococcus aureus</i> and Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), Methicillin-resistant <i>Staphylococcus epidermidis</i> (MRSE), <i>Enterococcus faecalis</i>, <i>Escherichia coli</i> and <i>Candida albicans</i>. Certain compounds inhibit bacterial growth with low MIC values (μg/ml).

ME 1036, formerly CP5609, is a novel parenteral Carbapenem with a 7-acylated imidazo [5, 1-b] thiazole-2-y1 group directly attached to the Carbapenem moiety of the C-2 position and evaluation against the clinical
isolates of gram-positive and gram-negative bacteria. ME 1036 displayed broad activity against aerobic gram-positive and gram-negative bacteria. Unlike other marketed β-lactam antibiotics, ME 1036 maintained excellent activity against multiple-drug-resistant gram-positive bacteria, such as Methicillin-resistant *Staphylococci* and Penicillin-resistant *Streptococcus pneumoniae* (Mizuyo Kurazono, et al., 2004).

The ethyl gallate purified from a dried pod of Tara (*Caesalpinia spinosa*) intensified β-Lactam susceptibility in Methicillin-resistant and Methicillin-sensitive strains of *Staphylococcus aureus* (MRSA and MSSA strains, respectively). The maximum activity of alkyl Gallates against MRSA and MSSA strains occurred at 1-nonyl and 1-decyl gallate, with an MIC at which 90% of the isolates tested were inhibited of 15.6 μg/ml (Hirofumi Shibata, et al., 2005).

The antibacterial activity of XRP2868, a new Streptogramin composed of a combination of RPR132552 (Streptogramin A) and RPR202868 (Streptogramin B), was evaluated against a collection of clinical gram positive isolates with characterized phenotypes and genotypes of Streptogramin resistance. The species tested included *Staphylococcus aureus*, Coagulase-negative *Staphylococci*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, and other species of *Streptococci*. The strong activity of factor A of the oral Streptogramin enabled the combination to be very potent against Streptogramin susceptible *Staphylococci*, *Streptococci*, and *Enterococcus faecium* (MICs = 0.03 to 0.25 μg/ml) (Michel Dupuis and Ronald Leclercq, 2006).

The preparation of Polyacrylate Nanoparticles in which an N-thiolated β-Lactam antibiotic was covalently conjugated onto the polymer framework. These Nanoparticles are formed in water by emulsion polymerization of an acrylated antibiotic pre-dissolved in a liquid acrylate monomer (or mixture of co-monomers) in the presence of sodium dodecyl sulfate as a surfactant and potassium per sulfate as a radical initiator. The emulsions have potent *in vitro*
antibacterial properties against Methicillin-resistant *Staphylococcus aureus* and have improved bioactivity relative to the non-polymerized form of an antibiotic. Additionally, the antibiotic properties of the Nanoparticles can be modulated by changing the length or location of the acrylate linker on the drug monomer (Edward Turos, *et al.*, 2007).

The rise in the rates of glycopeptide resistance among *Staphylococcus aureus* isolates is concerning and underscores the need for the development of novel potent compounds. Ceragenins CSA-8 and CSA-13, cationic steroid molecules that mimic endogenous antimicrobial peptides and examined against the clinical isolates of Vancomycin-intermediate *Staphylococcus aureus* (VISA), heterogeneous Vancomycin-intermediate *Staphylococcus aureus* (hVISA), as well as Vancomycin-resistant *Staphylococcus aureus* (VRSA). They also examined the concentration-dependent activity, inoculum effect, post antibiotic effect (PAE), and synergy in combination with various antimicrobials (Judy N. Chin, *et al.*, 2007).

The photodynamic antibacterial properties of a closely related series of phenothiazinium dyes were tested against several pathogenic strains of *Staphylococcus aureus*, four of which were methicillin-resistant. Illumination of the photosensitisers at a fluence rate of 1.75 mW cm$^{-2}$ generally resulted in the enhancement of antibacterial activity in liquid culture and in greater efficacy than the methicillin analogue flucloxacillin. For methylene blue, dimethyl methylene blue and new methylene blue illumination led to increase in bactericidal activity =16-fold, typically 4-fold. In addition, dimethyl methylene blue and new methylene blue were active against epidemic strains of methicillin-resistant *Staphylococcus aureus* at concentrations lower than that of Vancomycin (=0.5 µM) (Wainwright, *et al.*, 1998).

### 3.8. Treatment by Plant Drugs

Sitafloxacin is a new quinolone active against multi-resistant Gram-positive pathogens. An open study was conducted in patients with serious systemic infections with MRSA or Vancomycin-Resistant *Enterococci* (VRE).
Patients with MRSA were recruited if treatment with glycopeptides had failed. Of 11 patients with MRSA infection, four were cured, six failed treatment and one was indeterminate. Of nine patients with VRE infection (one patient had both pathogens), five were cured and four failed. Fifteen adverse events in twelve patients were potentially related to the study drug. Sitafloxacin was effective in VRE and some recalcitrant MRSA infections (Shetty, et al., 2000).

Tanaka, et al., (2002) have identified the 16 flavonoids from *Erythrina variegata* for their antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Erycristagallin and Orientanol B were identified as leading compounds for phytotherapeutic agents against MRSA infections and exhibited the highest activity with MIC values of 3.13 – 6.25 µg/ml⁻¹.

Ethanolic extracts of five traditional Australian medicinal plants were investigated for their abilities to inhibit clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* (VRE). Using plate-hole diffusion assays, the results were obtained. (a) extract from the leaves of *Eremophila alternifolia* (Myoporaceae) showed activity against MRSA; (b) extract from the leaves of *Acacia kempeana* (Mimosaceae) showed incomplete inhibition of VRE; (c) extracts from the leaves of *Amyema quandong* (Loranthaceae) and *Eremophila duttonii* (Myoporaceae) were active against both types of bacteria; (d) extract from the stem base of *Lepidosperma visidum* (Cyperaceae) was active against Methicillin-resistant *Staphylococcus aureus* (Enzo A. Palombo and Susan J. Semple, 2002).

In a continuing search for compounds with antibiotic activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) possessing multi-drug efflux systems, and demonstrated the activity associated with extracts from Southern prickly ash bark, *Zanthoxylum clava-herculis*. Bioassay-guided isolation of an alkaloid extract led to the characterization of the benzo[c]phenanthridine alkaloid chelerythrine as the major active principle. This compound exhibited potent activity against strains of MRSA, which were
highly resistant to clinically useful antibiotics via multi-drug efflux mechanisms (Simon Gibbons, et al., 2003).

Sato, et al., (2003) have reported that the five phytochemicals were isolated from *Erythrina poeppigiana* (Leguminosae) for antimicrobial activity against both Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*. Roots of *Erythrina poeppigiana* were macerated with acetone and the chloroform-soluble fraction of the residue was subjected to repeated silica gel column chromatography using various eluting solvents. Structures of the isolated compounds were determined by extensive spectroscopic studies. Spectral data indicated the presence of three different types of phytochemicals; isoflavonoids (Erypoegin A, Demethylmedicarpin and Sandwicensin), α-methyldeoxybenzoin (Angolensin) and Cinnamylphenol (Erypostyrene). Each compound was dissolved in dimethyl sulphoxide and added to agar plates (final concentration: 1.56 – 100 μg/ml⁻¹) and minimum inhibitory concentrations (MICs) were determined. While all compound showed anti-MRSA activity in this concentration range.

Fourteen extracts from Brazilian traditional medicinal plants used to treatment diseases were used to look for potential antimicrobial activity against multi-resistant bacteria of medicinal importance. *Staphylococcus aureus* strains were susceptible to extracts of *Punica granatum* and *Tabebuia avellanedae*. The minimum inhibitory concentrations (MICs) of the total extracts and of additional fractions of these plants were determined by employing strains of methicillin-resistant (MRSA) and –sensitive (MSSA) *Staphylococcus aureus*, including isolates of the PFGE clone A, which is prevalent in Brazil and two ATCC reference strains. Semi-synthetic furanonaphthoquinones (FNQs) showed lower MICs than those exhibited by natural occurring naphthoquinones. These natural products can be effective potential candidates for the development of new strategies to treat MRSA infections have been focused by Machado, et al., (2003).
The antimicrobial effect in vitro of aqueous and ethanolic extracts of garlic (*Allium sativum* Linn.), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn.) juice were assayed against *Staphylococcus aureus*; *Bacillus* spp and *Escherichia coli*. All the test organisms were susceptible to undiluted lime-juice. The aqueous and ethanolic extracts of garlic and ginger singly did not inhibit any of the test organisms. The highest inhibition zone of 19 mm was observed with a combination of extracts on *Staphylococcus aureus*. *Salmonella* spp were resistant to almost all the extracts except lime (Onyeagba, *et al.*, 2004).

Methanol extract of *Acorus calamus* L. rhizomes was investigated for its antimicrobial activities on various microorganisms including bacteria, yeasts and filamentous fungi. It exhibited high activity against filamentous fungi. However, it showed moderate activity against yeasts low activity against bacteria (Phongpaichit, *et al.*, 2004).

The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1 mg/l as required. The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of an organization after overnight incubation, this period extended for organisms such as anaerobes, which required prolonged incubation for growth (Jeffrey Andrews, *et al.*, 2005).

Hyeon *et al.*, (2005) have identified the antimicrobial activity of Berberine, the main antibacterial substances of *Coptidis rhizoma* and *Phellodendri cortex*, against the clinical isolates of MRSA, and the effects of berberine on the adhesion to MRSA and intracellular invasion into human gingival fibroblasts. Minimum inhibitory concentrations (MICs) of berberine against MRSA ranged from 32 to 128 μg/ml. Ninety percent inhibition of MRSA was obtained with 64 μg/ml or less of berberine.

Kabir, *et al.*, (2005) have concluded that the Six Nigerian medicinal plants *Terminalia avicennioides*, *Phylantus discoideus*, *Bridella feruginea*, *Ageratum conzoides*, *Ocimum gratissimum*, and *Acalypha wilkesiana* used by
traditional medical practitioners for the treatment of infections caused by Methicillin-Resistant Staphylococcus aureus (MRSA). Both water and ethanol extracts were effective on MRSA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the ethanol extracts of these plants ranges from 18.2 to 24.0 µg/ml and 30.4 to 37.0 µg/ml respectively. All the active plants contained at least trace amount of anthraquinones.

In 2005, Eileen Smith et al., have showed the antibacterial activity against MRSA in the crude hexane extract from the immature cones of Pinus nigra, was studied against several pathogens using disk diffusion method. It showed anti bacterial activity in several bacteria including Staphylococcus aureus. The anti-bacterial effect of A. continentals on MRSA is little known. CHCL soluble extracts of roots of A. continentals was found to exhibit distinctive anti-microbial activity at a level of 16 mg/ml (Seung-II Jeong, et al., 2006). The diterpene isopropimmaric acid was extracted from the immature cones of Pinus nigra (Arnold) using bioassay guided fractionization crude hexane extract iso pimaric acid was assayed against MDN and MRSA. The MIC was 32-64 mg/ml.

Ethanolic extracts and some fraction from 10 Indian medicinal plants known for anti bacterial activity were investigated for their ability to inhibit clinical isolates of β-Lactamase producing MRSA and MSSA. The extracts from the leaves of Ocimum sanctum showed better activity against the MRSA strain. The antibacterial potency of crude extracts was determined in terms of MIC by the tube dilution method, MIC values of the plant extracts, ranged from 1.3 to 8.2 mg/ml against the test bacteria (Farrukh Aqu, et al., 2005).

The antimicrobial activity of ethyl acetate, methanol and water extracts of Curcuma longa L against MRSA. The ethyl acetate extract was more active than the other extracts; the study examined whether the ethyl acetate extract could restore the anti bacterial activity of B-lactoms and alter the invasion of Human Mucosal Fibroblasts (HMFs) (Kang-Ju Kim, et al., 2005).
Ethanol, benzene, chloroform and aqueous extracts of *Picralima nitida* (seed, stem bark and root) were tested against five bacterial strains using the agar-well diffusion method. The ethanol extracts of the root and stem bark were active against 100% of the test organisms. Of the fifteen extracts treated, 40.0% were active against *Staphylococcus aureus* 20.0% each against *Pseudomonas aeruginosa* and *Escherichia coli* 33.3% against *Bacillus subtilis*. The MIC values for the ethanol extracts range from 6.25 to 50 mg/ml, while the MIC values for the cold water seed extract was 50 mg/ml. The results provide a rationalization for the traditional use of *P. nitida* for the treatment of various diseases (Nkere, *et al.*, 2005).

The effects of water and chloroform extracts of the leaves of *Lawsonia inermis* (henna plant) against the primary invaders of burnt wounds was investigated. Clinical isolates of *Staphylococcus aureus*, *Streptococcus* sp, *Pseudomonas aeruginosa*, *Candida albicans*, *Fusarium oxysporum*, and *Aspergillus niger* were treated with extracts of the leaves of *L. inermis* for antimicrobial activity using in vitro agar incorporation method and well diffusion methods respectively. The henna leaves extracts were able to inhibit the growth pattern of *Aspergillus niger*, *F. oxysporum*, *Streptococcus* spp. and *Staphylococcus aureus* were also inhibited by the extracts (Muhammad, *et al.*, 2005).

An ethnobotanical survey was carried out to collect information on the use of medicinal plants in Southern Western Ghats of India (Madurai District, Tamil Nadu) in the year of 1998-1999. A total of 60 ethnomedicinal plant species are distributed in 32 families. The plants used by Paliyar tribes are listed with Latin name, family, local name, parts used, mode of preparation and medicinal uses. The documented ethnomedicinal plants were mostly used to cure skin diseases, poison bites, stomach ache and nervous disorders (Ignacimuthu, *et al.*, 2006).

Chandrasekaran, *et al.*, (2006) have reported that the aqueous and methanol extracts from three mangrove plants viz., *Rhizophora mucronata*,
Rhizophora lamarkii and Bruguiera cylindrica were tested against 10 isolates of Methicillin - Resistant Staphylococcus aureus (MRSA) using disc diffusion method and evaluated the minimal inhibitory and minimal bactericidal concentrations. The mean zone of inhibition produced by the extracts against MRSA ranged from 8.4 to 22.7 mm. The minimum inhibitory concentrations were between 0.125 and 4 mg/ml, while the minimum bactericidal concentrations were between 0.25 and 8 mg/ml.

Antibacterial activity of 18 ethnomedical plant extracts were evaluated against nine bacterial strains (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, and Erwinia sp) and one fungal strain (Candida albicans). The methanol extract of Toddalia asiatica, Syzygium lineare, Acalypha frutcosa and Peltophorum pterocarpum could be potential sources of new antimicrobial agents compared to hexane extract. The collected ethnomedical plants were used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders (Veeramuthu Duraipandiyan, et al., 2006).

The antibiotic activity compounds against MRSA in chloroform extract of roots of Aralia continentalis was found to contain continentalic acid (CA) and dterpenic acid. The compound exhibited potent activity against standard (MRSA). It was determined that continentalic acid had MICs of approximately 8-16 mg/ml against Staphylococcus aureus (Seung-Ideeng, et al., 2006).

Antioxidant capacity and antimicrobial activities of Morchella conica Pers. extracts obtained with ethanol were investigated in this study. The antimicrobial effect of Morchella conica in ethanol extract was tested against six species of Gram-positive bacteria, seven species of Gram-negative bacteria and one species of yeast. The Morchella conica ethanol extract had a narrow antibacterial spectrum against tested microorganisms. The most susceptible bacterium was M. flavus. The crude extract was found active on
Staphylococcus aureus ATCC 25923 and Staphylococcus aureus Cowan I. The M. conica ethanol extract did not exhibit anti-candidal activity against Candida albicans (Turkoglu, et al., 2006).

The antimicrobial activity of aqueous, methanol, ethanol and ethyl-acetate leaf extracts of Bulbine lagopus (Asphodelaceae), Chironia baccifera (entianacea), Conyza scabrida (Asteraceae) and Dodonaeaviscosa var. angustifolia (Sapindaceae), were tested against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. In the disc-diffusion assay, 20 out of the 80 extracts showed activity. The best activity was observed in the ethanol extract of B. lagopus and the methanol extract of C. scabrida both having an MIC value of 0.3125 mg/ml (Thring, et al., 2007).

The antimicrobial potential of C. mellei against Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Mycobacterium smegmatis was determined by disk diffusion method and Minimum Inhibitory Concentrations (MIC) by two-fold serial dilution. C. mellei showed antimicrobial activity against Staphylococcus aureus and M. smegmatis in the disc diffusion method. Eight chemical compounds showed clear zones of inhibition out of this seven against Staphylococcus aureus. The ethyl acetate extracts have MIC value of 7.5 mg/ml against Staphylococcus aureus. Phytochemical tests indicated the presence of flavonoids, hydrolysable tannins, phytosterols and aromatic acids (Springfield, et al., 2006).

The n-butanol purified saponin extract of Sorghum bicolor were screened for anti-bacterial activity against three pathogenic microbes; Escherichia coli, Staphylococcus aureus and Candida albicans. The extract inhibited the growth of the Staphylococcus aureus. It was concluded that the saponins have inhibitory effect on gram-positive organism (Soetan, et al., 2006).

Four solvent extracts (methanol, ethanol, n-hexane and water) of Allium vineale L., Chaerophyllum macropodum Boiss and Prangos ferulacea L. were
investigated for its anti microbial activity against *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella enteritis* and *Salmonella typhimurium* by using disc diffusion method. The methanol, ethanol and n-hexane extracts of all the plants showed antibacterial activity against *B. cereus*, *B. subtilis*, *M. luteus* and *S. aureus*, while the methanol extract of *Allium vineale* was also active against *P. mirabilis* (Hisamettin Durmaz, *et al.*, 2006).

The aqueous and organic solvents extracts of *Boscia angustifolia* were screened for antibacterial and phytochemical properties. Alkaloids and saponins were detected in aqueous and chloroform extracts. These extract fractions were significantly (p<0.05) active against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumoniae* ranging from 10 to 120 mg/ml. The hexane and petroleum ether extracts did not show activity on the bacterial organisms used. The MIC of the aqueous and chloroform extracts was 20 and 10 mg/ml respectively (Hassan, *et al.*, 2006).

Ethanol and aqueous extract of *Heracleum sphondylium subsp. artvinense* was investigated for their antimicrobial activities against eight bacterial species (*Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Shigella*, *Streptococcus pyogenes*, and *Corynobacterium diphtheria*) and two yeast (*Candida albicans* and *C. krusei*). Both ethanol and aqueous extract of *H. sphondylium subsp. artvinense* showed antimicrobial activity against the gram-positive bacterium including *Staphylococcus aureus* (Ergene, *et al.*, 2006).

Methanolic extracts of six marine algae belong to *Rhodophyceae*, *Phaeophyceae* and *Chlorophyceae* from the North Aegean Sea (Turkey) were studied for their antibacterial activity against pathogenic microbes, 3 gram positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus* and *Enterococcus*
faecalis) and two gram negative bacteria. (Escherichia coli, Enterobacter aerogenes) Extracts of all the test marine algae except C. officinalis showed inhibition against *Staphylococcus aureus*. On the other hand, highest inhibitory activity among all the extracts was shown to *E. aerogenes* by *C. officinalis*. The extract from *C. barbata* has shown broader activity spectrum against all the test organisms (Taskin, et al., 2007).

Different concentration of oils obtained from two plants species belonging to family Fabaceae i.e. *Trigonella foenum-graecum* and *Pongamia pinnata* were evaluated for their antifungal and antibacterial activity against *Aspergillus niger*, *Aspergillus fumigatus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by MIC determination and dry-weight method. Both the oils showed high degree of antimycotic and antibacterial activity. *Pongamia pinnata* oil was more effective as compared to oil of *Trigonella foenum-graecum*. *Aspergillus niger* and *Staphylococcus aureus* were more sensitive to oil of *Pongamia. pinnata*. (Pritee Wagh, et al., 2007).

Aqueous-methanolic extract of *H. sabdariffa* was investigated for its phytochemical con-stituents, antimicrobial activity and cytotoxicity using brine shrimps lethality assay. The extract was found to contain cardiac glycosides, flavonoids, saponins and alkaloids. It exhibited antibacterial activities against *Staphylococcus aureus*, *Bacillus stearothermo-philus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas fluorescence* (Olaleye, et al., 2007).

The ointment batches containing different concentrations of the *D. theifolia* extract was applied topically to both infected and uninfected wounds inflicted on rats and the rate of wound closure assessed by wound area measurement. In vitro, the *D. theifolia* extract inhibited the different clinical wound isolates of *Staphylococcus aureus* with MICs ranging from 3.0 mg/ml for 3 of the 5 clinical strains of *Staphylococcus aureus* tested. Phytochemical studies show that crude *D. theifolia* stem contains saponins, tannins,
glycosides, flavonoids, terpenoids, carbohydrates, alkaloids and steroids (Odimegwu, et al., 2007).

Ethanol, methanol, chloroform, petroleum ether and aqueous extracts of leaves of Parrotia persica were evaluated for antibacterial activity. The zone of inhibition varied from 13 to 22 mm. The highest inhibition was obtained in methanol and ethanol extract. Chloroform and petroleum ether extracts did not show any inhibitory activity. The MIC value of the methanol extract for the test bacteria ranged between 3.12 and 6.25 mg/ml and that of ethanol extract ranged between 6.25 and 12.5 mg/ml. The results scientifically validate the use of this plant in the traditional medicine of Iran (Mohammad Ahanjan, et al., 2007).

Antimicrobial activity was observed in six endemic plant species, including Campanula lyrata subsp. lyrata and Abies nordmanniana subsp. bornmuelleriana plants. The MIC of C. lyrata subsp. lyrata (leaf and flower) extract was found to be 29 mg/ml for Baccillus subtilis and 4.5 mg/ml for Staphylococcus aureus, and the MIC of Abies nordmanniana subsp. bornmuelleriana (leaf) extract was found to be > 3.14 mg/ml against Bacillus subtilis and when minimum bactericidal concentration results were evaluated, it was observed that the plant extracts had bactericidal effects (Mehlika Benlil, et al., 2008).