Introduction
1. INTRODUCTION

Increasing incidence of multiple resistances in human pathogenic microorganisms in the recent years, largely, due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Resistance to antimicrobial agents is a major global public health problem (Lorenzo Drago, et al., 2007). Many infectious microorganisms are resistant to synthetic drugs, so an alternative therapy is very much needed to prevent the infections (Swapana Latha and Kannabiran, 2006).

Resistance is a natural response to antimicrobial stress based on the selection which weakens the effect of chemotherapy. The introduction of large numbers of chemotherapeutic agents into clinical practice has generated strains of microorganisms that survive and multiply in vivo in the presence of high drug concentrations. *Staphylococcus aureus* is a common, virulent pathogen associated with a variety of infections, ranging from moderate to severe, in the community as well as in the nosocomial setting. *Staphylococcus aureus* has extensive genomic variability and easily acquires tools for resisting against antimicrobials, in particular against β-Lactam antibiotics (Goretti Mallorqui, et al., 2004).

Until now, many β-lactams have been developed and widely used for chemotherapy for bacterial infections. However, the emergence of antibiotic-resistant strains of *Staphylococci*, in particular, *Staphylococcus aureus*, has been reported in many countries. Several previous reports have been suggested that the increasing prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) all over the world and those strains are prone to cause serious outbreaks (Mizuyo Kurazono, et al., 2004).

Over the last four decades, Methicillin-Resistant *Staphylococcus aureus* (MRSA) caused major problems in hospitals throughout the world and become highly endemic in many geographical areas (Kabir, et al., 2005; and Michelle Thouverez, et al., 2003). Classically, MRSA has been a nosocomial problem
associated with long hospital stays, numerous or prolonged antibiotic courses, the presence of invasive devices and proximity to an already infected or colonized patients (Sara and Carol Moore, 2002).

According to the latest report from the National Nosocomial Infection Surveillance System (NNIS), approximately 60% of all Staphylococcus aureus nosocomial infections in intensive care units (ICUs) were Methicillin resistance in 2003, representing as 11% increase in resistance compared to the previous five year period (Flayhart, et al., 2005). And also the NNIS reported that the proportion of hospital intensive care units in which Staphylococcus aureus is resistant to Methicillin from 35.9% in 1992 to 64.4% in 2006 (Judy, et al., 2007). In Guru Tegh Bahadur (GTB) Hospital, Delhi, 33% Staphylococcus aureus infections were found to be caused by MRSA, while the nasal carriage rate of this organism in health care workers was reported to be 39% (Sonal Saxena, et al., 2003).

The two million annual nosocomial infections in the United States approximately includes 260,000 are due to Staphylococcal aureus. The percentage of nosocomial Staphylococcus aureus isolated that are Methicillin-resistance Staphylococcus aureus has become establish over side the hospital environment and is now appearing in community population without identifiable risk factors (Mary Barber, et al., 1961).

1.1. Staphylococcus aureus

1.1.1. Morphology and Cultural Characteristics (Jawetz, et al., 1982)

Staphylococci are first observed in human pyogenic lesions by Von Recklinghausen in 1871. The members of the genus Staphylococcus are placed in Stomatococcus and the family Micrococcus. Staphylococci are gram positive cocci (0.5 to 1.5μm in diameter) that occur singly and in pairs, tetrads, short chains (three or four cells), and irregular "grape-like" clusters. They are nonmotile, nonspore forming usually Catalase positive, and typically unencapsulated or with limited capsule formation.
Fig. 1 Gram Stain of *Staphylococcus aureus* in Pustular Exudate

*Staphylococcus aureus* can grow on ordinary media within a temperature range of 10 - 42°C, the optimum being 37°C and the pH of 7.4-7.6 and they are aerobes and facultative anaerobes.

On Nutrient agar medium, the colonies are large (2-4 mm diameter), circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains of *Staphylococcus* produce golden yellow pigment, and some may be white, orange or yellow. The colonies on blood agar are similar to those on nutrient agar and produce β-haemolytic colonies. On MacConkey agar medium, the colonies are small, pink in colour due to lactose fermentation.

The strains of *Staphylococcus aureus* exhibit the following characteristics, these includes,

- Facultative Anaerobe
- Coagulase Positive
- Ferment Mannitol
- β-haemolysis on Blood agar
- Produce Golden yellow pigment
- Liquefy Gelatin
- Produce Phosphatase
Produce thermostable nuclease

1.1.2. Classification of Staphylococci

The Genus Staphylococci belongs to

- Domain: Bacteria
- Kingdom: Eubacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae
- Genus: Staphylococcus
- Species: aureus

The genus Staphylococcus contains 32 species, 16 of which are found in humans. The identification of Staphylococcal species is based upon the Coagulase production and it is performed to separate *Staphylococcus aureus* from other species that are collectively referred to as,

1) Coagulase - Positive Staphylococci and
2) Coagulase - Negative Staphylococci (Schleifers, et al., 1986).

**Coagulase - Positive Staphylococci**

1. *Staphylococcus aureus*
2. *Staphylococcus intermedius*
3. *Staphylococcus hylicus*
4. *Staphylococcus coagulas*

**Coagulase - Negative Staphylococci**

1. *Staphylococcus epidermidis*
2. *Staphylococcus saprophyticus*
3. *Staphylococcus cohnii*
4. *Staphylococcus novobiosepticus*
5. *Staphylococcus sciuri*
6. *Staphylococcus auricularis*
7. *Staphylococcus capitis*
8. *Staphylococcus urealyticus*
9. *Staphylococcus hominis*
10. *Staphylococcus warneri*
11. *Staphylococcus pasteuri*
12. *Staphylococcus haemolyticus*.

*Staphylococcus* species can be identified on the basis of a variety of conventional phenotypic characters. In addition, Species can be identified on the basis of molecular phenotypic properties such as cellular fatty acids, multilocus enzyme electrophoresis, and whole cell polypeptides and genotypic properties such as chromosome restriction fragments, macro restriction patterns and ribotypes.

1.2. Antibiotic Resistance in *Staphylococcus aureus* (Ananthanarayan and Jayaram Paniker, 2005)

Genes expressing antibiotic resistance in *Staphylococcus aureus* can be either in chromosomal or in Plasmid DNA. The first evidence of *Staphylococcus aureus* resistance to Penicillin appeared in 1941, only after 2 years since its introduction in clinical therapy. Penicillin resistance is plasmid mediated, and therefore, it spread out very quickly to several other strains, with the result that in the 1980s approximately 90% of *Staphylococcus aureus*, isolated from patients, were resistant. Unlikely, Methicillin-resistance is chromosome mediated, and therefore its diffusion is slower than the penicillin resistance. But the occurrence of Methicillin resistance is growing constantly. Because of the extended use and misuse of antibiotics, the number of bacteria that are resistant to antimicrobial agents is rapidly increasing (Pesavento, *et al.*, 2007).
Antibiotic resistance is acquired by *Staphylococcus aureus* through (Jonathan, et al., 2005)

1. Extra Chromosomai Plasmids carrying Resistance Genes.
2. Transposons carrying Additional Genetic Information and
3. Mutations in Chromosomai Genes.

Antibiotic resistance has increased rapidly during the last decade, creating a serious threat to the treatment of infectious diseases. Data from the Canadian Nosocomial Infection Surveillance Program have revealed that the incidence of MRSA, as a proportion of *Staphylococcus aureus* isolates,
increased from 1% in 1995 to 8% by the end of 2000 (John Conly, *et al.*, 2002).

**1.2.1. Penicillin Resistance in *Staphylococcus aureus***

Originally, *Staphylococci* are uniformly sensitive to penicillin, though occasional strains from the preantibiotic era have been found retrospectively to be capable of producing Penicillinase. Soon after Penicillin came to be used clinically, resistant strains began to emerge, first in hospital and then largely in the community.

Penicillin resistance is of three types,

- *Staphylococci* produce beta lactamase (Penicillinase) which inactivates penicillin by splitting the beta lactam ring. They produce four types of penicillinases, A to D. Hospital strains usually produce type A Penicillinase. Penicillinase is an inducible enzyme and its production is usually controlled by plasmids which are transmitted by transduction or conjugation. The same plasmids may carry genes for resistance to a range of other antibiotics and heavy metals.

- Changes in bacterial surface receptors, reducing binding of beta-lactam antibiotics to cells. This change is normally chromosomal in nature and it is expressed more at 30°C than at 37°C. This resistance also extends to cover beta lactamase resistant penicillins such as Methicillin and Cloxacillin. Some of these strains may show resistance to other antibiotics and heavy metals and cause of outbreaks of hospital infection. These strains have been called ‘epidemic methicillin-resistant Staphylococcus aureus or EMRSA.

- Development of tolerance to penicillin, by which the bacterium is only inhibited but not killed.
1.2.2. Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in the early 1960's from European Countries. *Staphylococci* resistant to Methicillin (or) Oxacillin should be generally regarded as resistant to all β-Lactams since the end of the 1970s and the occurrence of MRSA has increased steadily. Methicillin, the first of the semi synthetic Penicillinase-resistant penicillin, was introduced in 1959 to target strains of Penicillinase-producing *Staphylococcus aureus*. MRSA was 'born' at the moment it acquired the Methicillin-resistant gene mecA, a 2.1-kb exogenous DNA fragment, by horizontal transfer. The genes have been analyzed as mecA which encodes the low affinity penicillin binding protein PBP2' (Marta Aires and Herminia, 2004; Mark, *et al.*, 2002).

1.2.2.1. mec DNA

Approximately 30 to 50 kb of additional chromosomal DNA in the *Staphylococci*, mec region is not found from the susceptible strains of *Staphylococci* but it is present in Methicillin-Resistant strains. mec region contains mecA, the structural gene for penicillin-binding protein 2a (PBP2a). The mecI and mecR1 are regulatory elements controlling mecA transcription (Henry, 1997).

1.2.2.2. mecA gene & PBPs

Resistance in MRSA is related to a chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein called as PBP2a. These are membrane bound enzymes, which targets for all β-Lactam antibiotics. PBP2a has a decreased affinity for binding β-Lactam antibiotics resulting in resistance not only to methicillin but also to all β-lactams including penicillin and Cephalosporins. The mecA gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to various antibiotics (Adebola Onanuga, *et al.*, 2005).
PBP2a, a bacterial cell wall synthetic penicillin-binding protein with low-affinity binding to β-Lactam antibiotics (Yuki Katayama, et al., 2005) and the Methicillin resistance in *Staphylococcus aureus* is mediated by the production of an altered penicillin-binding protein, PBP 2a. The meca gene complex regulates the production of PBP 2a. Detection of the meca gene or of PBP 2a appears to most accurately detect Methicillin resistance in *Staphylococcus aureus* (Louie, et al., 2000).

The meca gene encodes the PBP2a (also termed PBP 2'), an inducible 76-kDa PBP that determines Methicillin resistance. There is no meca homolog in susceptible strains. Both susceptible and resistant strains of *Staphylococcus aureus* produce four major PBPs, PBPs 1, 2, 3, and 4, with approximate molecular masses of 85, 81, 75, and 45 kDa. PBPs are membrane bound DD-peptidases that have evolved from serine proteases, and their biochemical activity is mechanistically similar to that of the serine proteases. These enzymes catalyze the transpeptidation reaction that crosslinks the peptidoglycan of the bacterial cell wall. β-Lactam antibiotics are substrate analogs that covalently bind to the PBP active-site serine, inactivating the enzyme at concentrations that are approximately the same as the MICs. PBPs 1, 2, and 3, which have high affinity for most β-lactam antibiotics, are essential for cell growth and for the survival of susceptible strains, and binding of β-lactams by these PBPs is lethal. In methicillin-resistant cells, PBP 2a, with its low affinity for binding β-Lactam antibiotics can substitute for the essential functions of high-affinity PBPs at concentrations of antibiotic that are otherwise lethal (Henry, 1997).

In resistant isolates, PBP2a, that has low affinity for β-Lactam-insensitive transpeptidation activity that is crucial for the synthesis of an intact cell wall. PBP2a is encoded by meca, a gene residing on a 30 kb to 50 kb DNA fragment (mec DNA) unique to Methicillin-resistant isolates that is inserted at a specific chromosomal site. Genetic and molecular analyses of mec DNA have revealed that some isolates contain two additional genes,
mecR1 and mecl, located upstream of mecA and divergently transcribed from it (Vijay, et al., 1998).

In addition to their resistance against all β-lactam antibiotics, MRSA strains may be resistant to several other classes of antibiotic, including the aminoglycosides, quinolones, clindamycin and erythromycin. Therefore, infections caused by these strains are serious and difficult to treat (Durmaz, et al., 1997).

1.3. Pathogenicity and Virulence (Ananthanarayan and Jayaram Paniker, 2005)

*Staphylococci* produce two types of diseases. These includes,

1. Infections
2. Intoxications

In infections, the cocci enter into the damaged skin, mucosal or tissue sites, colonize by adhering cells or extracellular matrix, evade host defense mechanisms, multiply and cause tissue damage.

In intoxications, the disease is caused by the bacterial toxins produced either in the infected host or preformed *invitro*. The virulence factors described include the following:

1.3.1. Cell Associated Polymers

The cell wall polysaccharide peptidoglycan confers rigidity and structural integrity to the bacterial cell. It activates complement and induces release of inflammatory cytokines.

- Teichoic acid, an antigenic component of the cell wall facilities adhesion of the cocci to the host cell surface and protects them from complement – mediated opsonisation.

- Capsular polysaccharides surrounding the cell wall inhibits opsonisation.
1.3.2. Cell Surface Proteins

- Protein ‘A’ Present on most *Staphylococcus aureus* strains has many biological properties, including chemotactic, antiphagocytic and anticomplementary effects. It also induces platelet damage and hypersensitivity.

- Clumping factor, another surface protein is the ‘bound Coagulase’ which is responsible for Coagulase test.

1.3.3. Extracellular Enzymes

- Coagulase is an enzyme which brings about clotting of human or rabbit plasma. It acts along with a ‘Coagulase reacting factor’ (CRF) present in plasma, binding to prothrombin and converting fibrinogen to fibrin.

- Lipases or lipid hydrolases produce by *Staphylococci* which help them in infecting the skin and subcutaneous tissues.

- Hyaluronidase breaks down the connective tissue. Staphylokinase (Fibrinolysin), fatty acid modifying enzymes and proteases help in initiation and spread of infection.

- Nuclease or a heat stable nuclease is a characteristic feature of *Staphylococcus aureus*.

- *Staphylococci* possess Protein receptors for many mammalian proteins such as fibronectin, fibrinogen, IgG and C1q. These facilitate Staphylococcal adhesion to host cells and tissues.

1.3.4. Toxins from *Staphylococcus aureus*

1.3.4.1. Cytolytic Toxins

Cytolytic toxins are membrane-active substances, consisting of four hemolysins and a leucocidin. These includes,
Hemolysins

Alpha Hemolysin - It is a protein inactivated at 70°C, but reactivated paradoxically at 100°C. It lyases rabbit erythrocytes, but it is less active against sheep and human red cells. It is also toxic to macrophages, lysosomes, muscle tissues, renal cortex and the circulatory system.

Beta Hemolysin - It is a Sphingomyelinase, hemolytic for sheep cells, but not for human or rabbit cells. It exhibits a 'hot-cold phenomenon', the haemolysis being initiated at 37°C, but becoming evident only after chilling.

Figure - 2 Virulence determinants of Staphylococcus aureus
Gamma Hemolysin - It is composed of two separate proteins, both of which are necessary for hemolytic activity.

Delta Hemolysin - It has a detergent-like effect on cell membranes of erythrocytes, leucocytes, macrophages and platelets.

**Leucocidin**

Leucocidin (Panton-Valentine toxin) is also a two component toxin, like gamma lysin, being composed of two components (S and F). The bicomponent membrane – active toxins as the Staphylococcal leucocidin and gamma lysin have been grouped as *synergothymenotrophic toxins*.

**1.3.4.2. Enterotoxin**

Enterotoxin is responsible for the manifestations of Staphylococcal food poisoning – nausea, vomiting and diarrhea 2-6 hours after consuming contaminated food containing preformed toxin. The toxin is relatively heat stable. Eight antigenic types of Enterotoxin are currently identified, named as A, B, C1-3, D, E and H. They are formed by toxigenic strains, singly or in combination. The toxin also exhibits pyrogenic, mitogenic, hypotensive, thrombocytopenic and cytotoxic effects.

**1.3.4.3. Toxic Shock Syndrome Toxin (TSST)**

Toxic shock syndrome (TSS) is a potentially fatal multi system disease presenting with fever, hypotension, myalgia, vomiting, diarrhea, mucosal hyperemia and an erythematous rash which desquamates subsequently. This is associated with infection of mucosal or sequestered sites by TSST-producing *Staphylococcus aureus* strains usually belonging to bacteriophage group I. Staphylococcal Enterotoxins and TSST-1 are super antigens which are potent activators of T-lymphocytes. This leads to an excessive and dysregulated immune response with release of cytokines interleukins 1, 2, tumour necrosis factor and interferon gamma. This explains the multi system
involvement and florid manifestations in Staphylococcal food poisoning and Toxic shock syndrome.

1.3.4.4. Exfoliative (Epidermolytic) Toxin

Exfoliative toxin is also known as ET or ‘exfoliation’ and it is responsible for the ‘Staphylococcal scaled skin syndrome’ (SSSS), Exfoliative skin diseases in which the outer layer of epidermis gets separated from underlying tissues. The severe form of SSSS is known as Ritter's disease in the newborn and toxic epidermal necrolysis in older patients.

1.4. Clinical Significance

Staphylococcal infections are among the most common of bacterial infections and range from the trivial to fatal. Staphylococcal infections are characteristically localized pyogenic lesions. As a nosocomial pathogen, Staphylococcus aureus has been a major cause of morbidity and mortality. The Common Staphylococcal infections are the following:

- Skin and Soft Tissue - Folliculitis, Furuncle (Boils), Abscess (Particularly Breast abscess), Wound infection, Carbuncle, impetigo, Paronychia and Cellulitis
- Musculoskeletal - Osteomyelitis, Arthritis, Bursitis and Pyomyositis
- Respiratory - Tonsillitis, Pharyngitis, Sinusitis, Otitis, Bronchopneumonia, Lung abscess, Empyema, rarely Pneumonia
- Central Nervous System - Abscess, Meningitis, Intracranial thrombophlebitis
- Endovascular - Bacteremia, Septicemia, Pyemia and Endocarditis
Staphylococcus aureus is capable of producing "distant" diseases, which are mediated by the secretion of toxins. The toxins can be producing directly by bacterial colonization of the skin or mucosal surfaces or indirectly by microorganisms present in the food or beverages. The direct route is exemplified by the Staphylococcal Scalded Skin Syndrome (SSSS), which is due to mucosal or wound colonization by Staphylococcus aureus producing Exfoliative toxin A (or) B, and also by the Staphylococcal Toxic Shock Syndrome (TSS), related to the production of Toxic Shock Syndrome Toxin (TSST), or Exotoxin B (or) C, the indirect route is by food intoxication.

In this case, the toxin is ingested with contaminated dish and disease follows shortly there after in the form of vomiting and diarrhea. Food intoxication is due to Staphylococcal toxins called Enterotoxins and they are
heat stable. Cooking may kill the organisms but does not denature the toxins (Me Comick, et al., 2001).

Images of Staphylococcal infections (http://www.healthhype.com/staph-skin-infections.html)

Fig. 4 Abscess is an infection characterized by a collection of pus underneath a portion of the skin

Fig. 5 Cellulitis is an infection of the deeper skin tissue, which appears as a red, swollen, warm, tender skin patch of various sizes (Cellulitis on a shoulder in a child).

Fig. 6 Impetigo is a crust-forming Staph infection of the skin, mainly occurring in pre-school children
Fig. 7 Folliculitis is a skin condition caused by an inflammation of one or more hair follicles.

Fig. 8 Furuncle develops from infected hair follicle, when adjacent skin tissue is involved. Most commonly it appears on the neck.

Fig. 9 Paronychia is an infection of the skin folds of the nails.

Fig. 10 Scalded Skin Syndrome (SSS) is extensive red rash, like scald, caused by toxins released by Staphylococcus aureus.
The presence of *Staphylococcus aureus* in foods can produce a potential public health hazard, since many strains of *Staphylococcus aureus* produce the enterotoxins. The most common symptoms of Staphylococcal food poisoning include vomiting and diarrhea, which occurs 2 to four hours after ingestion of the toxin (the illness may be relatively mild lasting only a few hours) but some cases may require hospitalization. Foods commonly associated with Staphylococcal food poisoning are meat, meat products, salads, cream-filled bakery products and dairy products (Chambers, 2003).

Three patterns of Carriers are distinguished

1. Persistent Carriers
2. Intermittent Carriers
3. Non-Carriers

Approximately 20% of healthy people are persistent carriers, 60% are intermittent and 20% are non-carriers. Most infants become colonized shortly after birth, but carriage decreases with age. In many people the pattern of carriage changes between the ages of 10 to 20 years.

*Staphylococci* are also can transmit from person to person upon transmission the organism may become established as part of the recipients normal flora and later be introduced to sterile sites by trauma or invasive procedures. Alternatively the organism may be directly introduce into normally sterile sites, such as by a surgeon or nurse during surgery. The person to person of *Staphylococci* particularly those that have acquired antimicrobial resistance, most notably occurs in hospitals and presents substantial infection control problems (Von Eiff, *et al.*, 2001).

1.5. Emergence of MRSA

In the recent years, antimicrobial resistance in bacteria has been considered as a major problem in public health. Incidence of community-acquired and hospital-acquired *Staphylococcus aureus* infections has been
rising with increasing emergence of drug-resistant strains referred as Methicillin - Resistant *Staphylococcus aureus* (Arch, et al., 2006).

There are two important types of MRSA infection

1. Hospital-acquired MRSA (HA-MRSA)
2. Community-acquired MRSA (CA-MRSA)

- The first and mostly recognized type of MRSA infection is caused by HA-MRSA. These infection are nosocomial usually involve in multi-drug resistant strains and are nearly associated with toxic shock syndrome.

- The newer type of MRSA infection is caused by CA-MRSA, which involves strains that are resistant only to beta-lactam agents and may be associated with toxic shock syndrome.

MRSA has emerged over the past 30 years as an important cause of hospital infections and is actually an organism that is frequently transmitted in hospitals and prenatal units. An outbreak causing several pathological problems to the newborns and mothers, the MRSA is considered a public health problem in neonatology because of its strong potential for dissemination in the wards, high levels of antibiotic resistance, associated with high rates of morbidity and mortality.

MRSA had caused major problems in hospitals throughout the world. Infection caused by MRSA isolates have increased greatly during the last decades in the hospitals. During the late 1970’s strains of *Staphylococcus aureus* resistant to Methicillin and Gentamycin were increasingly responsible for many outbreaks in USA and Great Britain. Recent exports indicated that MRSA strains increased for 10 to 40% of *Staphylococcus aureus* isolated from European hospitals.

MRSA is an emerging community pathogen. It was first reported in the early 1990s among closed communities of Aborigines in Western Australia. Outbreaks of CA-MRSA infections in healthy children and adults have been
described worldwide. CA-MRSA infections tend to occur in younger persons than do Hospital - Acquired Methicillin - Resistant *Staphylococcus aureus* (HA-MRSA) infections. They often cause sporadic cases of skin and soft tissue infections but cases of necrotizing pneumonia.

In Taiwan, MRSA has been noted only since the early 1980's however two third of infections were acquired in the hospital. Over 1 - 25 million population in the southern part of Ireland have increased in new isolates of MRSA since 15 in 1992, 58 in 1993, 92 in 1994 and 100 in 1995. A continuing increase in number of isolates is reported from Irish hospitals each year. Prevention of cross infection and further spread of endemic strains requires effective control measures (Cotter, *et al.*, 1997).

In Central Europe (Austria, Germany and Switzerland), the prevalence of MRSA increased from 1.7% in 1990 to 8.7% in 1995. Data from the SENTRY surveillance study reported a nosocomial prevalence of MRSA of 34.2% in the USA, 26.3% in Europe and 34.9% in Latin America during 1997-1999, and 40.4% in South Africa, 66.8% in Japan and 22.4% in Australia during 1998-1999 (Marta Aires and Herminia, 2004).

MRSA is also emerging in the community particularly in the United States. Where 28% of community acquired *Staphylococcus aureus* strains may be resistant to Methicillin. From 2 million annual nosocomial infection in the United States approximately 2, 60,000 are due to *Staphylococcus aureus*. The percentage of nosocomial infection by *Staphylococcus aureus* isolates are Methicillin resistance increases from 14.3% in 1987 to 39.7% in 1997.

In the United States, the prevalence of MRSA and Methicillin-resistant Coagulase-negative *Staphylococci* (MR-CoNS) steadily increased in the 1990s, and in 2000 the rate of resistance of *Staphylococcus aureus* to Methicillin was 55.3%. A nationwide survey carried out in Japan during 1992 and 1993 showed that 60.3% of 7,033 *Staphylococcus aureus* isolates were resistant to Methicillin, and 86% of those were isolated from inpatients. In addition, clinical infections caused by Vancomycin-resistant *Staphylococcus aureus*
isolates carrying the vanA gene was reported from the United States in 2002 (Mizuyo Kurazono, et al., 2004).

In humans, the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) largely depends on the region, site of infection, and weather the infection was nosocomial or community onset. The prevalence of MRSA isolated from nosocomial infections was greater than that from community infections, and spanned from 1.8% in Switzerland and 2% in The Netherlands, to 73.8% in Hong Kong. In Europe, prevalence of MRSA is increasing: from 0.1-1.5% for Denmark, Sweden and The Netherlands, to 30.3-34.4% for Spain, France and Italy. In the United States, the prevalence of methicillin resistance among nosocomial *Staphylococcus aureus* extracts increased from 2.1% in 1975 to 35% in 1991 and it is greater than in community *Staphylococcus aureus* isolates (Pesavento, et al., 2007).

In the United States and in some European countries, MRSA accounts for 10 to 40% of all *Staphylococcus aureus* isolates. Increased surveillance, including screening of high-risk patients, has been recognized as an important component of effective infection control programs to limit the spread of MRSA in hospitals (Louie, et al., 2000). In USA, the National Nosocomial Infections Surveillance (NNIS) system reported a 51.3% Methicillin rate among *Staphylococcus aureus* strains from 18397 intensive care unit patients between January 1998 to June 2002, which corresponds to an increase of 25% relative to the rates reported for 1995-1999 (Marta Aires de Sousa, and Herminia de Lencastre, 2004).

In 2004, the National Nosocomial Infections Surveillance (NNIS) system report has identified methicillin resistance in 59.5% of *Staphylococcus aureus* infections in intensive care unit patients. This report represented an 11% increase in resistance compared with rates for the period 1998 to 2002. In hospitalized pediatric patients with *Staphylococcal* infections, the proportion expressing methicillin resistance increased from 10% in 1995 to 29% in 2001.
MRSA has recently emerged as an important cause of community-acquired infection in many parts of the United States (Helio, et al., 2007).

Initially, MRSA nosocomial infections were mainly detected in large tertiary hospitals and in intensive care units, where colonized and infected patients as well as colonized health care workers were a significant source of cross-infection. Currently, MRSA is one of the most common pathogens in hospitals of all sizes worldwide (Marta Aires and Herminia, 2004). Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an increasingly common cause of nosocomial infections, causing severe morbidity and mortality worldwide, and accounting in some hospitals for more than 50% of all *Staphylococcus aureus* diseases (Lorenzo Drago, et al., 2007).

1.6. Microbiological & Molecular Diagnosis

Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in clinical samples continues to be an important, since infections due to MRSA have high morbidity and mortality rates. Methods to detect MRSA in clinical samples ideally should have high sensitivity and a short time to reporting the results (Heiman Wertheim, et al., 2001).

Reliable and rapid methods to identify these organisms are crucial in any clinical laboratory. The tube Coagulase test is regarded as a gold standard. But an alternative approach is to incorporate chromogenic substrates into a suitable isolation medium for the identification of *Staphylococcus aureus* isolates (John Merlino, et al., 2000).

Various techniques based on biochemical and molecular features have been applied to investigate MRSA spread including biotyping, antibiotic susceptibility patterns, immunoblotting, peptidoglycan analysis and bacteriophage typing. Recent approaches to typing MRSA exploit its molecular characteristics that include plasmid analysis, restriction fragment length polymorphism (RFLP), ribotyping and pulsed-field gel electrophoresis (PFGE).
Polymerase chain reaction (PCR), finger printing techniques have been used for the epidemiological typing of many organisms (Cotter, *et al.*, 1997).

Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other method of testing, or when disc diffusion methods are not appropriate. The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1mg/L as required. The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after overnight incubation (Jennifer, 2001).

In predicting the effectiveness of an antibiotic against a microorganism, in-vitro tests of varying complexity are used. They range from agar diffusion sensitivity tests, through determination of minimum inhibitory concentration (MICs), to estimation of minimum bactericidal concentration (MBC). In general, MBC determinations are laborious and are reserved for special clinical situations, most notably bacterial endocarditis, where there is good evidence that a lethal effect is essential for effective treatment (Clarence, *et al.*, 1985).

The *invitro* determination of bacterial susceptibility to antimicrobial agents is commonly done by disk diffusion. More quantitative determination of growth-inhibiting activity (minimal inhibitory concentration; MIC) is accomplished by agar or broth dilution and determination of bacterial-killing activity (minimum bacterial concentration; MBC) (Kwang and Bascom, 1981).

1.7. Treatment by Antibiotics

The development of antibiotics for control of pathogenic bacteria has been of imperative need in this era of drug-resistant infections (Edward Turos,
et al., 2007). *Staphylococcus aureus* has a great adaptive power to antimicrobial agents, and little by little it has been acquiring resistance to all antibiotics available in clinical practice. Due to an increasing number of infections caused by multidrug-resistant Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains, therapy has become problematic. Many MRSA are susceptible only to Glycopeptide antibiotics and investigational drugs (Marta Aires and Herminia, 2004)

In the last several years, the new antimicrobials specifically targeted against Gram-positive bacteria or MRSA strains have been launched. But the strains with decreased susceptibility to these antibiotics have been increased and therefore unfortunately, development of new antimicrobial agents in next future seems to be declining (Lorenzo Drago, et al., 2007).

Methicillin - Resistant *Staphylococcus aureus* (MRSA) strains are increasingly encountered and cannot be treated with available β-Lactams. Most of the Methicillin - resistant (and also some Methicillin - susceptible *Staphylococcus aureus* [MSSA]) strains are resistant to all available quinolones and Vancomycin - Intermediate (VISA) and Vancomycin - Resistant *Staphylococcus aureus* (VRSA) strains have appeared (Kim Credito, et al., 2007).

The occurrence and proliferation of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a cause for major concern not only in the clinical environment but also in community life. In fact, MRSA is resistant to virtually all kinds of β-Lactam. Although Vancomycin and teicoplanin are the two glycopeptides presently used in clinics for the treatment of multi-resistant infections by gram-positive organisms, Vancomycin-resistant *Staphylococcus aureus* has arisen in the United States (Hirofumi Shibata, et al., 2005).

The rise of Multidrug resistance has prompted renewed interest in the development of novel antimicrobial agents. It is not surprising that pathogenic species have adopted survival mechanisms to counteract both old and new antimicrobials. The drive to produce newer agents targeting novel sites that
may circumvent resistance is critical to the long-term control of bacterial infection. One frequently studied target is the bacterial membrane (Judy, et al., 2007).

The spread of resistance to antibiotics undermines the therapeutic utility of anti-infective drugs in current clinical use. Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains appeared in the hospital environment after introduction of the semi-synthetic penicillin named as Methicillin, leaving Vancomycin as the last line of defense for MRSA treatment. *Staphylococcus aureus* organisms intermediately susceptible to Vancomycin were first isolated in 1997 in Japan and later in other countries. With in recent appearance of Vancomycin-resistant clinical isolates, no antibiotic class is effective against multi-resistant *Staphylococcus aureus* infections (Magally Romero, et al., 2006).

Cefepime is a novel broad-spectrum Cephalosporin with potent activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains due to strong affinity for *Staphylococcus aureus* penicillin-binding proteins (PBPs), including PBP 2A, the additional protein responsible for Methicillin resistance mechanism (Cedric Jacqueline, et al., 2007).

The glycopeptides have been considered as the drugs of choice for the treatment of severe MRSA infections. Linezolid has recently been recommended as an alternative treatment for some of those infections. The requirement for effective new agents to treat MRSA infections is becoming increasingly apparent due to the emergence of strains with reduced susceptibility to glycopeptides. New agents including Tigecycline, daptomycin and Ceftobiprole have recently been introduced into clinical practice for resistant gram-positive infections (Olivier Denis, et al., 2006).

Daptomycin is a cyclic lipopeptide derived from *Streptomyces roseosporus*. The mode of action is unique in that it binds to bacterial membranes, in the presence of physiological levels of calcium ions. It is primarily effective against Gram-positive bacteria, due to its inability to
penetrate the outer membrane of Gram-negative organisms. It is active against a wide range of multi-drug resistant (MDR) organisms for which there are very few therapeutic alternatives, such as MRSA and VRE (Helio, et al., 2007).

1.8. Treatment by Plant Drugs

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better compatibility with the human body (Jinga Parekh, et al., 2005).

Globally, about 85% of the traditional medicines have used for primary healthcare are derived from plants. India is one of the twelve mega-biodiversity countries of the world having rich vegetation with a wide variety of plants with medicinal value. Herbal medicines have good values in treating many diseases including infectious diseases (Ignacimuthu, et al., 2006).

Resistance to antimicrobial agents is prevalent among many of these bacteria in numerous geographical locations worldwide. Of particular concern is the proliferation of the nosocomial pathogen Methicillin-resistant Staphylococcus aureus (MRSA) prompted by poor infection control procedures and antibiotic selection pressure. Antimicrobial drug resistance is also of economic concern with impact on doctors, patients, health-care administrators, pharmaceutical companies and the public. The development of new antimicrobial drugs has been used to overcome resistance. However, given that plant-derived medicines have been part of traditional health-care in most of the world and the antimicrobial properties of plan-derived compounds
is well-documented, there is an increasing interest in plants as sources of antimicrobial agents (Enzo and Susan, 2002).

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Veeramuthu Duraipandiyan, et al., 2006).

Plants belonging to Leguminosae are known to produce numerous secondary metabolites (Phytochemical), some of which function in defense systems against pathogenic fungi and bacteria. Phytochemical with both antifungal and antibacterial activity would provide insight into the development of new therapeutic agents against mixed MRSA infections (Sato, et al., 2003).

Three different plants are used for this present work. They are *Acorus calamus*, *Tridax procumbens* and *Pisum sativum*. Out of these plants, *Acorus calamus* is a medicinal plant, *Tridax procumbens* is shrub which is present in most of these places, and *Pisum sativum* is food additive. In our work three different plant parts are used, they are seeds, whole shrubs and rhizome of *Pisum sativum*, *Tridax procumbens* and *Acorus calamus* respectively.

1.9. Botanical Information for Sweet flag

Scientific Classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
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<tr>
<td>Family</td>
<td>Acoraceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Acorus</em></td>
</tr>
</tbody>
</table>
Species : calamus

Common Names

English : Sweet flag
Hindi : Bacc, Gorbacc
Tamil : Vasampu

1.9.1. Morphology and Distribution of the Plant

It is cultivated in areas up to 1,800m. In marshes also cultivated. Semi-aquatic monocot, rhizomatous perennial herb, rhizome creeping much branched as thick as the middle finger, cylindrical or slightly compressed, light brown or pinkish brown externally, white and spongy within leaves bright green, distichous ensiform, base equitant, thickened in the middle margins wavy, flowers light brown densely packed in sessile cylindric spadix, fruits oblong turbinate berries with a pyramidal top, seeds few, pendant from the apex of the cells. Rhizome is the useful part. It has the glycosoid, acorin. The rhizome also has essential oil containing calamen, calamenol and asarone (Vidyaratnam, 2002).

Probably indigenous to India, Acorus calamus is now found across Europe, in Southern Russia, Northern Asia Minor, southern Siberia, China, Japan, Burma, Sri Lanka, Southern Canada and Northern USA.

1.9.2. Uses

The rhizome of Acorus calamus is acrid and bitter. It has thermogenic, laxative, carminative, anthelmintic, expectorant and insecticidal properties. It is used as a nervine tonic and as a sedative.

The rhizome is used in the treatment of nephropathy, cough, bronchitis, inflammation and gout. It is used in the treatment of mental disorders such as epilepsy, delirium, amentia, and depressions. This drug is also used to
treat skin diseases and dysentery. It has been used in the treatment of snake-bite. The rhizome is made into a powder and it is used for eliminating lice.

Scented leaves and rhizomes which have been used medicinally, for its odor, and as a psychotropic drug. It is known by a variety of names, including cinnamon sedge, flagroot, gladdon, myrtle flag, myrtle grass, myrtle sedge, sweet cane, sweet myrtle, sweet root, sweet rush, and sweet sedge:

The morphological distinction between the *Acorus* species is made by the number of prominent leaf veins. *Acorus calamus* has a single prominent midvein and then on both sides slightly raised secondary veins (with a diameter less than half the midvein) and many, fine tertiary veins. This makes it clearly distinct from *Acorus americanus*.

The leaves are between 0.7 and 1.7 cm wide, with average of 1 cm. The sympodial leaf of *Acorus calamus* is somewhat shorter than the vegetative leaves. The margin is curly-edged or undulate. The spadix, at the time of expansion, can reach a length between 4.9 and 8.9 cm (longer than *A. americanus*). The flowers are too longer, between 3 and 4 mm. *Acorus calamus* is infertile and shows an abortive ovary with a shriveled appearance.

1.9.3. Chemistry

Calamus has been an item of trade in many cultures for thousands of years. Calamus has been used medicinally for a wide variety of ailments and its smell makes calamus essential oil, valued in the perfume industry.

In antiquity in the Orient and Egypt, the rhizome was thought to be a powerful aphrodisiac. In Europe *Acorus calamus* was often added to wine, and the root is also one of the possible ingredients of absinthe. Among the northern Native Americans, it is used both medicinally and as a stimulant; in addition, the root is thought to have been used as an entheogen among the northern Native Americans. In high doses, it is hallucinogenic.
The calamus has long been a symbol of male love. The name is associated with a Greek myth: Kalamos, a son of the river-god Maeander, who loved Karpos, the son of Zephyrus and Chloris. When Karpos drowned, Kalamos was transformed into a reed, whose rustling in the wind was interpreted as a sigh of lamentation.

The name Sweet Flag refers to its sweet scent (it has been used as a strewing herb) and the wavy edges of the leaves which are supposed to resemble a fluttering flag. In Japan, the plant is a symbol of the samurai's bravery because of its sharp sword-like leaves. Even now many families with young boys enjoy "Sweet Flag Bath (shōbu yu)" in the Boy's Festival (Tango no Sekku) on May 5. Also, the legendary Japanese sword Kusanagi was said to resemble a calamus.

### 1.9.4. Etymology of the word Calamus

Cognates of the Latin word Calamus are found in both Greek (kalamos, meaning "reed") and Sanskrit (kalama, meaning "reed" and "pen" as well as a sort of rice) — strong evidence that the word is older than all three languages and exists in their parent language, Proto-Indo European. The Arabic word qalam (meaning "pen") is likely to have been borrowed from one of these languages in antiquity, or directly from Indo-European itself.

From the Latin root "calamus", a number of modern English words arise: calamari, meaning "squid", via the Latin calamarium, "ink horn" or "pen case", as reeds were then used as writing implements; calumet, another name for the Native American peace pipe, which was often made from a hollow reed; shawm, a medieval oboe-like instrument (whose sound is produced by a vibrating reed mouthpiece); chalumeau register, the lower notes of a clarinet's range (another reed instrument).

The use of higher plants and preparation made from them to treat infections is an age old practice in a large part of the world population in developing countries where there is dependence on traditional medicine for a
variety of diseases. Interest in plants with anti-microbial properties has revived as a consequence of current problems associated with the use of antibiotics. (http://en.wikipedia.org/wiki/Sweet_Flag).

1.9.5. Traditional Medicinal Uses

Seeds are thought to cause dysentery when eaten raw. In Spain, flour is considered emollient and resolvent, applied as a cataplasm. It has been reported that seeds contain trypsin and chymotrypsin which could be used for contraceptive, ecbolic, fungistatic and spermicide. Significant amounts of toxicity or anti-metabolites in peas.

http://www.hort.purdue.edu/newcrop/cropfactsheets/pea.html

1.10. Botanical Information for Tridax daisy

1.10.1. Scientific Classification

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Asterales
Family : Asteraceae
Genus : Tridax
Species : procumbens

http://plants.usda.gov/java/ClassificationServlet?source=display&classid=TRPR5

Common Names

English : Coat buttons, Tridax daisy
Hindi : Ghamra
Tamil : Thata poodu
1.10.2. Morphology and Distribution of the Plant

*Tridax procumbens* has a low perennial, often rooting at the nodes. It has ovate, lobed, stiffly hairy leaves and solitary, erect flower heads. They are produced throughout the year and have cream ray florets and yellow disc florets. It is best known as a widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperate regions worldwide.

The plant bears daisy-like yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plumelike white pappus at one end. The plant is invasive in part because it produces so many of these achenes, up to 1500 per plant, and each achene can catch the wind in its pappus and be carried some distance. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates.

1.10.3. Medicinal uses

Fabrifuges; generally healing; stomach troubles, diarrhoea, dysentery.

http://en.wikipedia.org/wiki/Tridax_procumbens

http://www.hort.purdue.edu/newcrop/CropFactSheets/doob.html

1.11. Botanical Information for Garden Pea

1.11.1. Scientific Classification

<table>
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<tr>
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<tr>
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<td>Family</td>
<td>Fabaceae</td>
</tr>
</tbody>
</table>
Genus : *Pisum*
Species : *sativum*

**Common Names**

English : Garden pea, Peas
Hindi : Mathar, Bhattani
Tamil : Pattani, Vellaippatani

**1.11.2. Morphology and Distribution of the plant**

It is cultivated in higher altitudes. Annual herbaceous tendril climber with hollow stems, leaves are Pinnately compound, leaflets entire, stipules auricled, flowers white, red or pink, fruits straight or curved pods, 5-10 cm long, seeds globular, smooth, green, yellowish green, bluish green, white, grey brown, 6-9 per pod, round or flattened, sometimes wrinkled. Edible Parts are Leaves and Seeds. The seeds are sweet, acrid, cooling, lactative, depurative, emollient and resolvent. They are useful in vitiated conditions of pitta, burning sensation, skin diseases, leprosy, cough, measles, cuts and wounds (Vidyaratnam, 2002).

**1.11.3. Uses**

In early times, peas were grown mostly for their dry seeds. In modern times however peas are usually boiled or steamed which breaks down the cell walls and makes the taste sweeter and the nutrients more bio-available. New cultivars of peas were developed by the English during this time which became known as "garden peas" and "English peas." The popularity of green peas spread to North America. Thomas Jefferson grew more than 30 cultivars of peas on his estate. With the invention of canning and freezing of foods, green peas became available year-round, not just in spring as before.

http://en.wikipedia.org/wiki/Pea
The seed is contraceptive and fungistatic. The dried and powdered seed has been used as a poultice on the skin where it has an appreciable affect on many types of skin complaint including acne. The oil from the seed, given once a month to women, has shown promise of preventing pregnancy by interfering with the working of progesterone. The oil inhibits endometrial development. In trials, the oil reduced pregnancy rate in women by 60% in a 2 year period and 50% reduction in male sperm count was achieved.

http://gardenbed.com/source/49/4894_med.asp

In some parts of the world, dried peas are consumed split as dahl, roasted, parched or boiled. "Green peas are the number one processed vegetable specifically in UK and USA. Green foliage of garden pea is also used as vegetable in parts of Asia and Africa. Leaves are used as a pot herb in Burma and parts of Africa". Oil from ripened seed has antisex hormonic effects; produces sterility and antagonizes effect of male hormone. "Based on protein digestibility of peas in broilers, it is reported that the natural protein of peas and faba bean is almost entirely digested in the small intestine and the impaired performance in literature was attributed to an increased secretion of endogenous protein. "Cultivars such as 'Alaska' 'Super Alaska,' 'Supergreen,' and 'Alaska Wilt Resistant have long been the standard type of canning pea. Wrinkled-seeded garden peas are sweeter than smooth seeded types."