1. INTRODUCTION

1.1 *Staphylococcus aureus*

Staphylococcus aureus is a Gram positive bacterium which is non-moving small round shaped or non-motile cocci. *S. aureus* appears as grape-like (staphylo-) clusters due to which it is called Staphylococcus. It is commonly pronounced as "*S. aureus*" or "Staph aureus" in medical literature. *Staphylococcus* was first discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses in a knee joint. It belongs to the family *Staphylococcaceae* which affects all known mammalian species, including humans. *Staphylococcus* is among top five most common causes of infections caused after any injury or surgery in humans (**Ogston, 1984; Rayan, 2004)**.

1.1.1 Infections

Staphylococcus aureus may cause wide range of diseases, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacterimia (blood poisoning), and sepsis. Its prevalence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the most common causes of nosocomial infections which frequently cause post-surgical wound infections **(Rayan, 2004)**.

1.1.2 Treatment and antibiotic resistance

The infections caused by *S. aureus* are commonly treated by penicillin and its derivatives in most of the countries. However, penicillin resistance is exceptionally common, and first-line therapy is most usually a penicillinase-resistant β-lactam antibiotics (e.g. Oxacillin, cloxacillin, nafcillin). Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, but its use is controversial because of the high risk of damage to the kidneys **(Korzeniowski and Sande, 1982; Bayer *et al.*, 1998)**. The choice of drug and duration of treatment depends on the site of infection and on severity. Antibiotic resistance in *S. aureus* was infrequent when penicillin was first introduced in 1943. By 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant; and, by 1960, this had risen to 80%. Today, *S. aureus* has become resistant to many commonly

used antibiotics. **(Chambers, 2001)**. It expresses resistance to beta-lactam antibiotics by beta-lactamase production, methicillin resistance, penicillin tolerance, and deficiency in penicillin binding proteins (PBPs). The clinical implication of all these phenomena is not fully defined **(Lacey, 1984)**.

1.1.3 Methicillin-resistant *S. aureus*

Methicillin resistant *Staphylococcus aureus* commonly abbreviated as **'MRSA'** is one of the most notorious strains of *S. aureus* which have become resistant to most β-lactam antibiotics. It is responsible for several infections which are difficult to treat. Any strain of *S. aureus* which develops resistance to beta-lactam antibiotics, which include the penicillins and the cephalosporins are called as MRSA. The development of such resistance does not cause the organism to be more intrinsically virulent than strains of *Staphylococcus aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous. It is particularly troublesome in hospitals and nursing homes, where patients with open wounds, invasive devices, and compromised immune systems are more prone to infection than the common public **(Lowy, 1998; Ito and Hiramatsu, 2003)**. These strains are most often found associated with institutions such as hospitals, but are becoming increasingly prevalent in community-acquired infections **(Lacey, 1984; Chambers, 2001)**.

The original MRSA infections associated with exposure in the health care setting, particularly in hospitals are referred to as hospital-acquired MRSA (HA-MRSA) **(Klein *et al.*, 2007)**. In 1990s, a new strain of MRSA emerged in the community setting occurring among young healthy individuals with no exposure to the healthcare setting. The infections caused by these strains are called community-acquired MRSA (CA-MRSA) **(Beam and Buckley, 2006; David and Daum, 2010)**.

1.2 Community-Acquired MRSA (CA-MRSA)

Conventionally, MRSA strain has been contemplated a major nosocomial pathogen in healthcare facilities, but in the past decade, it has been observed emerging in the community as well. The first case of community- acquired MRSA (CA-MRSA) infection in the United States was reported in 1980 **(Saravolatz *et al.*, 1982; Huang *et al.*, 2006)**. More-widespread identification of CA-MRSA in the United States began in the 1990s, following the report of CA-MRSA infections among four children **(CDC, 1999)**. Since then, this community-acquired MRSA strain (CA-

MRSA) has quickly spread across the globe **(Zetola *et al.,* 2005; Kluytmans and Kluytmans, 2006; Klein *et al.,* 2007)**. Outbreaks of CA-MRSA have been reported among children **(Herold *et al.,* 1998)**, athletes **(MMWR, 2003)**, nurseries **(Otter and French, 2006)** and obstetrical wards **(Saiman *et al.,* 2003)**.

The CA-MRSA strains have been involved in skin and soft tissue infections including furuncles, abscesses, folliculitis, impetigo, cellulitis, and, more rarely, in cases of severe sepsis, necrotizing fascitis, and necrotizing pneumonia **(Monaco *et al.,* 2005)**. In addition to wide spread occurrence and incidence CA-MRSA strains appear to be virulent in particular. Invasion of CA-MRSA strain in the community comprises the fourth and latest wave of antibiotic resistance. Outbreaks of CA-MRSA now occur globally and with a similar epidemiology, even though the specific clones that have emerged vary with different geographical places. CA-MRSA strains are not only escapees from health care setting; their genotypes point out that they are not closely related to endemic hospital clones **(Chambers and DeLeo, 2009)**. The spread of resistant CA-MRSA strains across the globe is becoming more common and posing potential threat to the life of community. The CA-MRSA strain is commonly known as the *Staphylococcus aureus* subsp. *aureus MW2*.

1.2.1 Epidemiology of Community-Acquired MRSA

The origins of community-acquired strains of *S. aureus* are subject to debate. The epidemiology of CA-MRSA is quite similar regardless of country of origin. Two molecular markers not found in typical hospital MRSA are strongly associated with emergence of CA-MRSA apart from of geographical origin: a particular cassette element encoding *mecA* and genes encoding Panton-Valentine leukocidin (PVL). The presence of PVL (Panton-Valentine leukocidin) among CA-MRSA isolates is more variable **(Chambers and DeLeo, 2009)**. For example in Australia and the United Kingdom most CA-MRSA clones do not produce PVL **(Nimmo and Coombs, 2008; Rollason *et al.,* 2008)** and prevalence of PVL among the more common CA-MRSA isolates from Denmark ranged from 17% to 100% **(Larsen *et al*., 2009)**. The earliest reported cases of CA-MRSA infection in the US were caused by a USA400 strain, MW2. USA400 has been supplanted by USA300, which is by far the most frequent cause of CA-MRSA infections in the US **(CDC, 1999)**.

The Nasal carriage of MRSA has increased as a community pathogen. Approximately 30% of people have asymptomatic nasal colonization with *S. aureus*.

Between 2001 and 2004 carriage of MRSA strains in a US population based study approximately doubled from 0.8% to 1.5% and the percentage of community-associated MRSA genotypes increased from 7% to 24.2% **(Gorwitz *et al.*, 2008)**. USA300 has emerged as an easily transmitting strain compared to other strains **(Crum *et al.*, 2006)** which could account for increasing carriage rates in the community. Therefore, risk for CA-MRSA infection in any individual or group cannot be ruled out.

1.2.2 Treatment of Community-Acquired MRSA

The rapid emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has posed immense impact on empirical choice of antibiotics for infections caused by suspected staphylococci. This nosocomial pathogen is imposing potential threat to public-health. Mild CA-MRSA infections are curable in an out-patient department, but options for oral antibiotics are diminishing due to increased resistance levels of susceptibility which vary in different geographical locations. Most of the beta-lactam antibiotics, fluoroquinolones and clindamycin are no longer effective for treating the range of staphylococcus infections especially skin and soft-tissue infections.

In many regions where CA-MRSA skin and soft tissue infections are widespread, traditional antimicrobial treatment choices by fluoroquinolones and clindamycin are not justified, leaving physicians to depend on other feasible options with long-acting tetracyclines, trimethoprim-sulfamethoxazole (TMP-SMX), linezolid and vancomycin, which have severe impact of harsh adverse effects **(Powell and Wenzel, 2008; Chambers and DeLeo, 2009)**. In addition, adjunctive agents such as rifampin and fusidic are used in combination with above antibiotics (**Nathwani *et al.*, 2008)**. Although resistance to intravenously administered drugs remain little but treatment of CA-MRSA infections by antibiotics should be closely monitored depending on site and severity of infections.

1.3 *In silico* drug target identification through metabolic pathways analysis

The technological advances have revolutionized the process of novel potential drug target identification which aids in the drug discovery. The increasing availability of large number of complete microbial genome sequences has provided an opportunity for researchers to uncover all possible potential drug targets in

pathogens which have not yet been discovered. The availability of high-quality genome-wide metabolic reconstructions (**Oberhardt *et al.*, 2009**) presents a new approach for the rational and systematic identification of metabolic drug targets in a pathogenic organism. Functionally, biological pathways can be categorized into three classes i.e. metabolic pathways, gene regulation pathways and signal transduction pathways. Metabolic pathways are accountable for the chemical alterations involved in the biosynthesis or decomposition of various metabolites (e.g. carbohydrates, proteins, nucleic acids, lipids) (**Chavali *et al.*, 2012**). During the metabolic reconstruction process, there might be hundreds of putative metabolic enzymes whose structural and functional information is not yet identified by experimental methods. Annotation of proteins with metabolic information is mandatory for mapping the metabolic pathways on genomic data **(Freiberg**, **2001)**.

The biological function relies on the coordinated action of many molecular interactions of gene products and metabolites, systematically organized in so-called pathways (**Persidis, 1998; Ideker *et al.*, 2001; Pawson and Nash, 2003**). Any disturbance in the pathway flow or connections through slight alteration of one or more individual pathway components can lead to impaired functions (**Brown and Superti-Furga, 2003**). One of the most important advantages of the metabolic pathway analysis in pathogens are identification of novel potential drug targets, which can be exploited for novel drug discovery to combat the infections caused by life threatening pathogens. One of the recently adopted strategies is based on *in silico* comparative analysis of metabolic pathways of the host *Homo sapiens* and the pathogen, in which enzymes from the biochemical pathways of pathogen from the KEGG metabolic pathway database are compared by performing a BLASTp search against the non-redundant database restricted to the *H. sapiens* subset. This approach has been successfully employed in recent times to identify potential drug targets in several pathogens such as *Mycobacterium tuberculosis* (**Anishetty *et al.*, 2005**), *Helicobacter pylori* (**Dutta *et al.*, 2006**)*, Pseudomonas aeruginosa* (**Perumal *et al.*, 2007**), *Aeromonas hydrophila* (**Sharma *et al.*, 2008**)*, Clostridium perfringens* (**Chhabra *et al.*, 2010**). Similar methodology has been used to identify the novel putative drug targets in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

1.4 Structure based drug design approaches

Advancement in the genomics and proteomics technologies has revolutionized the computer-aided drug design (CADD) process, extending across almost all steps in the drug discovery development, from target identification to lead finding, and from lead optimization to pre-clinical trials (**Tang *et al.,* 2006**). Structure-based drug design (SBDD) aims to identify chemical compounds that bind strongly to active site of biologically relevant molecules, e.g. enzymes or receptors, for which three-dimensional structures are known. The designed ligands should be able to inhibit or stimulate the biological activity of their target. With the advancement in 3D structural determination techniques such as nuclear magnetic resonance, X-ray crystallography and even homology modelling, structure-based design of ligands or inhibitors has appeared as a vital tool in drug discovery (**Zeng, 2000; Anderson, 2003)**.

The most commonly used methods of structure based drug design are virtual screening and *de novo* ligand designing. The virtual screening is carried out by the docking method in which small or huge ligand databases are screened for a target. In *de novo* ligand designing, novel ligands are built-up within the constraint of active site of the target (**Yadav *et al.,* 2011**). SBDD is a robust tool, and is an integral part of the drug discovery pipeline today.

1.5 Justification of the work

The emergence and increasing prevalence of community acquired-methicillin resistant *Staphylococcus aureus* (CA-MRSA) strain that is resistant to available antibiotics demands the discovery of new therapeutic approaches. It has been reported that CA-MRSA may be replacing the traditional hospital-acquired MRSA (HA-MRSA). The spread of resistant CA-MRSA strains across the globe are becoming more common and posing potential threat to the life of the community. Because of multidrug resistance, particularly among CA-MRSA, alternative and effective therapeutic options are urgently needed. The availability of complete genome sequences of CA-MRSA, has paved a new way for identifying the novel drug targets. Through the complete genome analysis of the pathogen, it is possible to compile a list of potential gene products (proteins) and their functions which are non-homologous to the proteome of *Homo sapiens*. The novel potential drug targets can be identified through the metabolic pathways analysis in the community

acquired-methicillin resistant *Staphylococcus aureus*. The novel unique identified drug target(s) can be modelled by the molecular modelling techniques. Subsequently, modelled 3D structure of the target can be exploited for discovering the novel ligands or inhibitors so that the menace of antimicrobial resistance in CA-MRSA can be prevented.

1.6 Objectives of the study

The present research work was carried out with following objectives:

1. To select a candidate drug target out of putative drug targets for CA-MRSA (identified through metabolic pathways analysis).

2. To perform phylogenetic and *in silico* proteomic analysis of candidate drug target.

3. To perform homology modelling, dynamics studies and virtual screening for candidate drug target.