SUMMARY AND CONCLUSIONS

INTRODUCTION
One of the most notorious opportunistic pathogen with high mortality rate is Staphylococcus aureus. It is well known for its pandemic out breaks and also for its resistance to many known antibiotics. The non-resistant variants of S. aureus are easy to cure, whereas multi drug resistant (MDR) strains are comparatively typical to cure. As S. aureus have such lethal qualities which are hazardous to public health, Centers for Disease control and Preventions 2013 reports MRSA in the hit list of Super bugs. The infection caused by normal strain and by MDR, does not show much difference, which is a major challenge in treatment and hence in some cases, may cause life threatening conditions.

Globally around 19% deaths are reported due to various infectious diseases (Lozano et al., 2012) which accounts for the highest mortality rate among any other diseases. The major infectious and resistant bacteria are termed as ESKAPE pathogens by Rice (Rice, 2008). Alone in United States the mortality due to MRSA are higher compared to HIV and tuberculosis (Boucher and Corey, 2008).

The antibiotic resistance has made a great impact on the once-treatable infections, which now lacks proper treatment and cure. The trend of global antibiotic resistance may be directly interlinked to their use. Since the inception of antibiotics way back in 1940s it is being continuously over consumed for treatment. Due to which the bacterial population undertake the natural phenomenon of survival of fittest and undergo resistance. The MDR being a major problem, is now slowly demolishing the golden era of antibiotics as even the last resort of antibiotics is also failing in treating infections.

In pre-antibiotic era the mortality rate by S. aureus infections was 82% in healthy individuals where as 98% in age group of >50years (Skinner, 1941). Although the modern era of antibiotics has combated many bacterial infections, yet about 20-30% healthy individuals are carriers of S. aureus (Wertheim et al., 2005). The onset of penicillin resistant strains of S. aureus in 1940s, has simultaneously paralleled the emergence of MRSA, and many other antibiotic resistant strains (McDonald, 2006).
The present day treatment of MRSA infections are combated by broad spectrum antibiotic vancomycin, but the recent studies suggests that even Vancomycin strains have emerged (Appelbaum, 2007).

*S. aureus* being a gram-positive bacterium, the cell wall components include capsule or polysaccharide slime layer, teichoic acid, protein A (SPA) and peptidoglycan layer. The peptidoglycan layer serves as a protective layer in the gram-positive bacteria (Scheffers and Pinho, 2005). The peptidoglycan layer holds many surface proteins which are easy to target either as vaccine candidate or drug targets. The surface protein, Protein A binds to the complement binding site of the host IgG binding protein thereby inhibiting the classical pathway of host (Romagnani et al., 1982). It also helps in maintaining osmotic stability and anchors many cell wall proteins that are capable in colonizing on host cells and inducing toxins. Such proteins are defined as MSCRAMM (J M Patti et al., 2003).

The first antibiotic resistant *S. aureus* was discovered in 1960, from then many new antibiotics were discovered but with time *S. aureus* acquired resistance. Thus, MDR is a major problem with many outbreaks by food poisoning and nosocomial infections worldwide in recent years. The present-day challenge is facing pandemic nature, MDR and taking precaution from acquiring staph infections from both hospital and community settings. These challenges have motivated to carry out the present work which deals with CADD, immunoinformatics and in vitro antibacterial studies. The main motto of our study was to identify therapeutic candidates that are common in all 44 strains whose sequences are publicly available. The availability of genome and proteome sequences was handy to carry out the task. Identifying common therapeutics enables physician for convenient and easy treatment of infections.
OBJECTIVES
The detailed literature survey and all other factors mentioned, has powered us to undertake the current study with following objectives:

- Systematic in silico screening of putative therapeutic candidates in Staphylococcus aureus.

- Structure based virtual screening, molecular docking and molecular dynamics studies to discover novel MurE inhibitors.


- Predicting promiscuous epitopes in SPA antigen of S. aureus for developing peptide base vaccines.

THESIS OUTLINE
With the above-mentioned objectives, the present thesis was outline into six chapters. The Chapter 1 deals with introduction and literature survey, Chapter 2 deals with identification of therapeutic candidates, Chapter 3 deals with discovering lead molecule, Chapter 4 deals with determining activity of lead molecule, Chapter 5 deals with predicting epitopes and Chapter 6 presents major conclusions.

MAJOR CONCLUSIONS
Staphylococcus aureus is a Super bug which is associated both with nosocomial and community mediated infections. The mortality rate by S. aureus is comparatively higher than some of the well-known dread full disease like HIV. The major drawback in treating Staph infections include: MDR, quick emergence of resistance to antibiotics and most importantly the infections caused by any strain show similar kind of symptoms. Hence, a common therapeutic target is very much essential for accurate therapy.

In the current study, for identification of drug targets some of the essential properties like the target must have definite assay methods to ensure high throughput screening, must possess 3D structure to ensure druggability, constant expression of target, should not possess any competitor, and essentially must be disease specific (Gashaw et al.,
were set as criteria. Based on the mentioned criterions, two best drug targets were commonly identified in all the strains of S. aureus namely UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase (MurE) and cell division protein FtsA. MurE was found to be druggable target and FtsA to be a novel drug target. The best common vaccine candidate includes Peptidoglycan binding protein, which satisfies the essential criteria like having antigenic property, ≤ 3 transmembranes, a domain with epitope that can bind host immunoglobulin.

MurE enzyme plays an important role in peptidoglycan biosynthesis pathway by adding L-lysine to the third position of the stem peptide. Hence being a very attractive drug target. The structure based virtual screening and molecular docking process against MurE have determined seven potential molecules that interact with active site residues of MurE. Of the seven the molecule LigPrep32109441 ((2R)-2-[[1-[(2R)-2-(benzyloxy carbonylamino)propanoyl]piperidine-4-carbonyl]amino]-5-guanidinopentan) was found to have the least docking score and binding affinity. The molecular dynamics simulation studies indicate that the protein ligand interaction complex formed is stable. The interactions at the end of the simulations were compared with that of reference frame indicates that the hydrogen bonds formed with active site residues Asp406 and Glu460 are highly stable. From this we can infer that the LigPrep32109441 is a potential lead molecule that can inhibit MurE enzyme.

The pharmacokinetic studies have suggested that the lead molecule possess drug-likeness properties, obeying Lipinski’s rule of five. The lead molecule has shown the antibacterial activity close to the standard drug vancomycin. The MIC of lead molecule at 30µg/mL was observed to be 3.75 µg/mL, with 34.44% of inhibition and IC₅₀ of 40.06µg/mL. These results suggest a promising lead molecule for developing a MurE inhibitor against treatment of S. aureus infections.

By employing immunoinformatics approach, two epitopes were identified in SPA antigen (Peptidoglycan binding protein) based on different physiological parameters. The docking studies suggests that the peptide sequence “NLNEEQRNG” of length nine residues may serve as the potent epitope for synthesizing a synthetic peptide vaccine. The current finding may further help the scientific communities and pharmacologists in developing a potent vaccine against staph infections.
FUTURE IMPLICATIONS

Cell division protein FtsA, is another proposed drug which can be studied for identification of novel inhibitors against it by virtual screening. The lead molecule identified as potential inhibitor of MurE by docking and *in vitro* AST methods can be further studied at molecular level (enzymatic assays). *In vivo* studies, of the lead molecule to determine its activity in infected mice and also to understand its mode of action. Furthermore, designing a carrier for lead molecule using nano particles, for targeted delivery. Biochemical assays on synthesis of peptides and their activity in *in vitro* and *in vivo* conditions could be determined for future development of vaccine against *S. aureus*. In the current study, a pipeline for identification of drug targets and vaccine candidates, was carried out manually. Automatizing this system would be of great deal for identification of therapeutic candidates in other pathogens. All these above-mentioned tasks, are some of the implications proposed for future.