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Research Article

SCREENING OF PUTATIVE THERAPEUTIC CANDIDATES IN SUPERBUG *(STAPHYLOCOCCUS AUREUS)*: A SYSTEMATIC *IN SILICO* APPROACH

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ABSTRACT

Objective: *Staphylococcus aureus*, a superbug and antibiotic resistant pathogen, is one of the most infection causing organism, ranging from skin allergies to severe lethal conditions. The prolonged use of different antibiotics and lack of optimal treatment over the antibiotic resistant species, led to the identification of new, better and promising therapeutic candidates.

Methods: A systematic *in silico* filtration process was employed, which includes subtractive channels and reverse vaccinology techniques.

Results: Here, we report 12 possible drug targets and two vaccine candidates based on essentiality, non-human homolog, virulent and localization, commonly in all the strains. Further characterization studies such as pathway analysis, chokepoint and structure prediction revealed, two proteins as the best drug targets one being novel and the other druggable. Only one protein has shown the characteristic feature of vaccine candidate, having antigenic property and an IgG binding domain.

Conclusion: 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, whereas the best common vaccine candidate includes Peptidoglycan binding protein. The therapeutic candidates reported in the present study might facilitate screening of new and better antimicrobial compounds, for an optimal treatment of *S. aureus* infections.

Keywords: *Staphylococcus aureus*, Drug target, Vaccine candidates, Subtractive proteomics, Reverse vaccinology.

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INTRODUCTION

Developing a novel drug against certain disease is a very intricate process with huge investments and also prolonged period of almost 12-15 years. The success of drug development mainly relies on the initial steps of drug discovery process which involves target identification and validation. Even clinically the failure of any drug mainly relies on whether it effectively acts on the target or on the safety issues of the drug [1]. Hence, target identification is very much essential, but the *in vitro* and *in vivo* processes are very costly and time-consuming which are eventually being replaced by modern computational methods. In the present study, we have taken the advantage of available proteomic data and other bioinformatics tools to screen the therapeutic candidates in a superbug *Staphylococcus aureus*.

S. aureus is a Gram-positive, facultative anaerobe, commensal, and opportunistic pathogen. It can survive in radical and adverse conditions such as temperature 7-48°C, pH 4.00-10.00 [2], high salt content, low a_w (water activity), and osmotic stress [3]. All these factors enable it to survive and colonize in anterior nares, gastrointestinal tract, groin, and axillae of humans [4]. The major transmission of *S. aureus* is by food poising [5], nosocomial routes and also from the community. It is capable of causing numerous diseases, ranging from minor skin infections to severe and lethal factors leading to death. These infections are most commonly treated by β-lactam antibiotics such as methicillin, penicillin, cephalosporins, and oxacillin, which mainly act on penicillin-binding proteins.

With a period, these bacteria have acquired methicillin resistance methicillin-resistant *S. aureus* (MRSA) that was first reported in 1961 [6] from the UK. The resistance was caused by *mecA* (methicillin resistance) gene acquired from distant species. This *mecA* gene is carried by *Staphylococcal* cassette chromosome *mec* mobile elements [7]. These strains have been evolved in two forms of infections hospital acquired-MRSA or nosocomial MRSA and community acquired-MRSA.

In the past decade, the mortality for drug resistance and antibiotics have very much increased by mainly six type of bacteria which are termed as *Enterococcus faecium*, *S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species pathogens by rice [8]. Of these six pathogens, one of the most notorious is MRSA and accounts in the superbug hit list according to the US, centers for disease control and preventions 2013 report. Although the antibiotic strains have reported in the 1960's, the increase in mortality rate, and infections have gone pandemic in past two decades with major outbreaks caused by food poisoning at the US in 1990 [9], Brazil in 1998 [10], Japan in 2000 [11], Austria in 2006 [12], and Paraguay in 2007 [13], and the number of cases filed are represented in a graph (Fig. 1). The survival of organism at varied temperatures and in unhygienic places makes it much favorable to attack in developing countries. India being a developing country with tropic climate has made the bacteria endemic. About 13,975 MRSA cases in 2008 and 12,235 MRSA cases in 2009 were reported at 15 different Indian tertiary centers by (Indian network for surveillance of antimicrobial resistance) [14]. These pandemic situations are needed to be controlled by new and novel therapeutic alternatives.

In the present day, the MRSA is treated with the broad spectrum antibiotics in one or more combinations, which include glycopeptides such as vancomycin and teicoplanin [15], sulfa drugs and daptomycin [16]. These drugs are still not up to the mark to completely cure the infections and unfortunately *S. aureus* is gaining resistance even to these therapeutics. One such glycopeptide, vancomycin drug is now not susceptible over MRSA, and a new strain vancomycin-resistant *S. aureus* has been evolved [17]. The evolvement of different strains and subspecies of *S. aureus* requires common drug target and vaccine candidates for alternative treatments. In the present day, the scenario for research on identification of drug targets and vaccine candidates mainly relies on academic research as the pharmaceutics has ceased such research due to lack of profits [18].

In drug discovery, the major task is concerned in identifying potential drug targets and by bench work, it takes a lot of time and money. To avoid these hurdles, we have taken the advantage of modern *in silico* approach which includes, screening the proteome of the pathogen for essential, non-human homolog, and virulent proteins. Later, they were characterized based on their function, cellular localization, and metabolic pathways. Here, we report the common drug targets and vaccine candidates from around 14 strains of *S. aureus* and 30 strains of its subspecies *S. aureus.* Identification of common drug targets might help the physicians to treat the infection with ease, and a single vaccine candidate against various strains may protect us from infections.

METHODS

In the present study for identification of putative drug target and vaccine candidate in different strains of *S. aureus,* we have applied systematic *in silico* screening approach, with different filtering phases. The first phase of the filter is to screen the proteome with subtractive proteomics approach which includes identification of essential, non-human homologs, and virulent proteins. The second filter predicts whether the proteins may act as possible drug targets or vaccine candidates by subcellular localization. The proteins localized in cytoplasm, extracellular, membrane, and cell surface are possible drug targets and those who are localized only on the cell surface are possible vaccine candidates. The third filter includes screening the putative drug targets based on the pathway and chokepoint analysis and for vaccine candidates based on antigenicity, domains capable of binding immunoglobulin proteins, and epitome prediction. Final filtering phase is for broad spectrum analysis and for non-human gut flora. The complete workflow of subtractive proteomics and reverse vaccinology is depicted in Fig. 2.

Data collection, databases, and tools employed

The prime motto of our study was to identify the common drug targets and vaccine candidates from different strains of *S. aureus* and its subspecies *S. aureus*. The complete set of protein sequences in FASTA format was retrieved from NCBI database [19]. The essential protein analysis was carried out using database of essential genes (DEG) [20], non-human homologs protein sequences by Human-BLASTP [21], virulent proteins were predicted by VirulentPred [22], the subcellular localization of essential, non-human homolog with virulent nature were predicted by CELLO [23] and PSORT [24].

The proteins that are localized in any part of the cell are subjected for drug target analysis and further characterized. To those who are localized only on the cell surface are analyzed and characterized for vaccine candidates. The possible drug targets were analyzed for their involvement in any of the known pathways of *S. aureus* by KAAS (KEGG automated annotation system) [25]. Similarly, these proteins were compared with human metabolic pathways. The chokepoints were analyzed, and then druggability or novelty of the target was done by BLASTP against drugbank targets [26].

The proteins that are localized on cell surface were analyzed, for antigenic property by Vaxijen 2.0 [27], presence of transmembrane helices by TMHMM [28], identifying the domains that have the capability to bind to immune cells of humans by domain search against InterProScan [29], and finally characterizing the vaccine candidate by identifying the epitopes by SVMTriP [30]. Then, broad spectrum analysis and non-human gut flora analysis were carried out for drug target and vaccine candidate by BLASTP option from Human Microbiome Project [31,32].

RESULTS AND DISCUSSION

With the increase in mortality rates due to *S. aureus* infection and its capability to undergo resistance and emerging new varieties of resistant strains have pledged this study. This study includes identification of common drug targets and vaccine candidates as a source for alternative therapeutics. In 2001, two MRSA strains (N315 and Mu50) were sequenced [33] and deposited in NCBI for the first time since then about 42 more strains were sequenced by various groups and made available for the public. The availability of such a huge data of genome and proteome of approximately 44 strains of *S. aureus* has enabled us to carry out the study on identification of common therapeutics among them.

From NCBI protein database, the complete proteome of 14 *S. aureus* strains and 30 *S. aureus* strains were retrieved. Of the 44 strains, 14 are annotated completely, 19 at contig level, and 11 are scaffolds. On an average genome size of every strain is about 2.8 Mb with approximately 2,700 proteins in each strain. The protein sequences from all the strains were retrieved on or before 18th August 2015, accounting to about 123,380 proteins.

The first phase of filtration in our study relies on the subtractive proteomic approach which is also known as differential genome display, proposed by Huynen *et al*. [34]. The main idea behind this paradigm was the fact that the parasitic microbes encode the lesser number of genes than that of free-living forms which make them pathogenic. The other point of this paradigm was that target must be a non-human homolog. During the course of time, this strategy was proposed; many scientists have successfully applied it, for mining the new therapeutic candidates. Some of the successful studies that included this strategy to establish novel therapeutics in *Plasmodium falciparum* [35], *Mycoplasma hyopneumoniae* [36], *Clostridium perfringens* [37], *Salmonella typhi* [38], *Neisseria* species [39], *Aeromonas hydrophila* [40], *Helicobacter pylori* [41], and in many other organisms.

In our study, we have first screened all the protein sequences against the proteins encoded by essential genes of *S. aureus* N315 and *S. aureus* NCTC8325 with the BLASTP parameters in DEG as E-value cutoff of 1E-05 and BLOSUM62 matrix. The proteins that are encoded by a minimum set of genes play a crucial role in the survival of the organism and are essential genes [42,43], with this basis we have identified the essential proteins. In the essentiality screening process on an average, approximately 730 proteins were predicted to be essential from each strain and, on the whole, essential proteins account for 19,041. These essential genes were then filtered based on the non-human homolog, as the target protein from the microbe should not have a homology with that of humans, as to reduce the probability of the drug acting on the human proteins [44]. This filtration was done by subjecting the obtained sequences to BLASTP against the human proteome with a stringent selection of having no hits. A total of 1,022 proteins have shown no homology with that of the human proteome, which approximately corresponds to 20-23 proteins per strain.

As the main goal of our study is to identify the common therapeutics against all the strains of *S. aureus*, we have manually mined the common and specific candidate proteins separately (Additional file (Tables S1-S3)). The virulence property for the common proteins from all

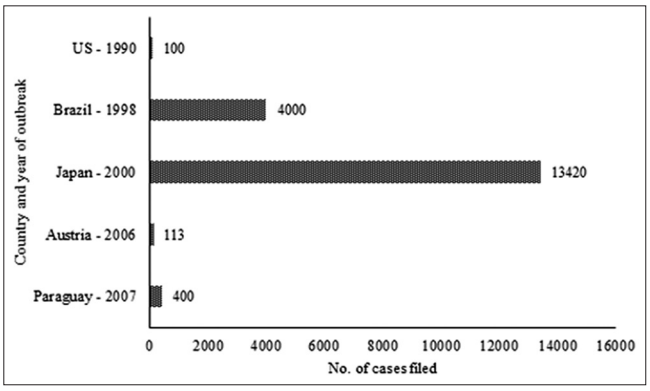

Fig. 1: Distribution of pandemics caused by *Staphylococcus aureus* in past decades

strains was identified by VirulentPred. As the target protein must have virulence character to induce disease for alternative therapy against microbes [45]. This analysis indicated that 14 proteins have virulence property. The cellular localization was identified by CELLO and PSORT, which indicated that only two proteins are present on the cell surface of the protein and others either in the vicinity of cytoplasm, extracellular or in the nucleus. The overall filtration is depicted in Fig. 3, which indicates the extraction of a minimum number of possible therapeutics from a pool of huge proteome.

From the second filtration, the proteins that are localized in cytoplasm, extracellular, and membrane are considered as possible drug targets and that which resulted to be localized only on the cell surface are considered as possible vaccine candidates. Then, these proteins are further proceeded to respective filtration process as shown in Fig. 2. The possible drug targets and vaccine candidates filtered based on essentiality, non-human homolog, virulence, and cellular localization are listed in Table 1.

The possible drug targets are then subjected to KAAS server to identify their role in the known metabolic pathways of *S. aureus.* This analysis indicated that all the 12 proteins are involved in different pathways and some in common pathways. Among them, seven proteins are found to be enzymes. Enzymes are one of the best and second largest classes [46] of targets in drug discovery. The majority of the proteins were found to be having a role in peptidoglycan synthesis followed by cell cycle proteins, phosphotransferase system (PTS) proteins, and proteins involved in xenobiotic degradations (additional file 2 [Table S4]). Further, the enzymes were subject to chokepoint analysis, whether the

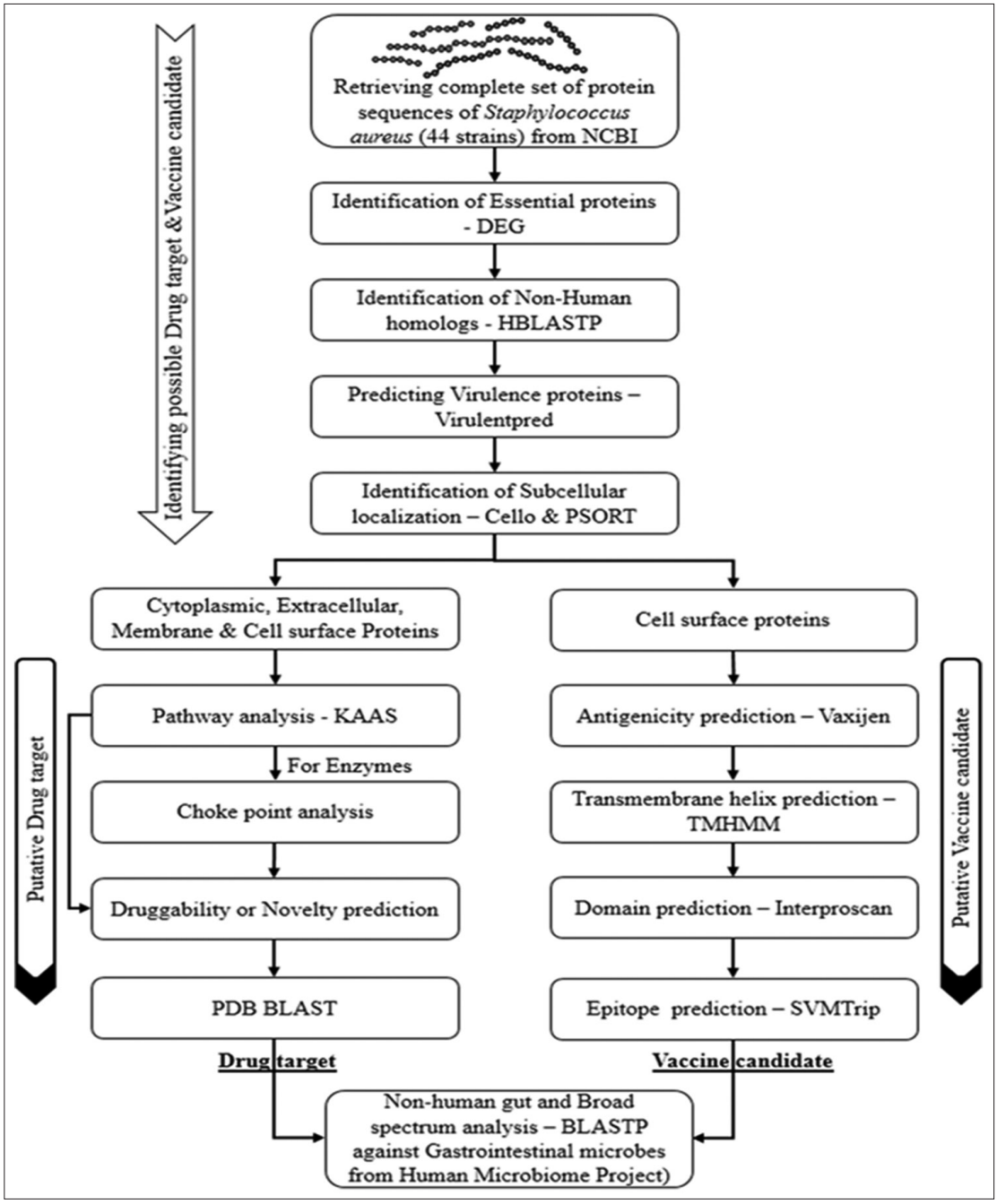

Fig. 2: Workflow for identification of drug targets and vaccine candidates

enzyme consumes a specific substrate or produces a unique product and balances the reaction [47]. The chokepoint reactions are observed for three enzymes and the reactions of the enzymes are:

a. PTS alpha-glucoside transporter subunit IIBC
   Protein EIIB N(pi)-phospho-L-histidine/cysteine + sugar = protein EIIB + sugar phosphate.
b. UDP-N-acetylglucosamine 1-carboxytransferase 1
   Phosphoenolpyruvate + UDP-N-acetyl-alpha-D-glucosamine = phosphate + UDP-N-acetyl-3-O-(1-carboxyvinyl)-alpha-D-glucosamine.
c. UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase
   ATP + UDP-N-acetyl-alpha-D-muramoyl-L-alanyl-D-glutamate + L-lysine = ADP + phosphate + UDP-N-acetyl-alpha-D-muramoyl-L-alanyl-gamma-D-glutamyl-L-lysine.

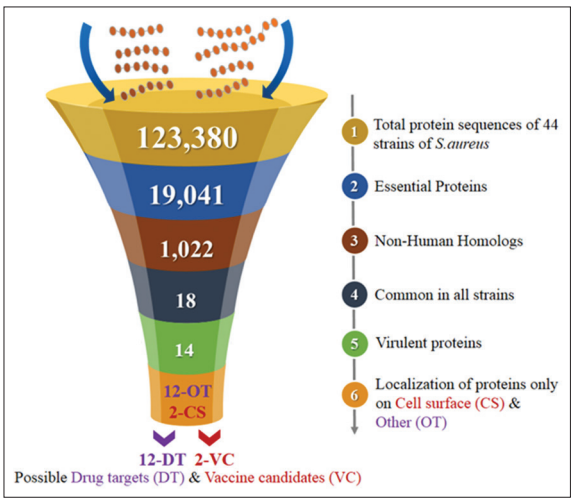

Fig. 3: Filtration for identification of possible therapeutic candidates

About five proteins were found to be druggable, and seven were found to be novel by drugbank analysis. The list of novel and druggable proteins are listed, with the drugs acting on the respective targets and the organism (Additional file 3 [Table S5]). Of the five druggable target proteins, four are enzymes. These set of 12 proteins were subjected to BLAST against Pluggable database (PDB) database to identify whether the targets have crystal structures. The 3-dimensional crystal structures of proteins define its biological activity and also define the topography of ligands interacting with the target proteins [48]. Hence, understanding the structure of proteins helps us in exploiting the selectivity and potency of the ligands. To only four protein targets the experimental structures are derived. Based on all the three filtrations, we could identify four common drug targets in all 44 strains of *S. aureus* (Table 2).

The two proteins that were found to be localized on the cell surface, N-acetyl mannosaminyl transferase and peptidoglycan binding protein were analyzed for having antigenic property by Vaxijen. The N-acetyl mannosaminyl transferase and peptidoglycan binding protein both have shown antigenic property score 0.4155 and 0.6982, respectively, which is greater than that for threshold value (0.4) for bacterial models and hence specifying it to be a probable antigen. Protein to be a valid vaccine candidate it should not have more than three transmembrane helices and should have a domain that can bind to the immunoglobulins of the host (humans). The TMHMM predictions revealed that there are no transmembrane helices in N-acetyl muramosaminly transferase, whereas peptidoglycan binding protein constitutes only one transmembrane helix ranging between 12 and 34 residues. The InterProScan have shown that

Table 1: List of proteins from primary and secondary filtration

Serial number	Protein ID	Protein name	Length	Filtration	
				Primary[a]	Secondary[b]
1	WP_000050762.1	PTS ascorbate transporter subunit IIA	147	Yes	Cytoplasmic
2	WP_000184370.1	Multispecies: Transglycosylase	301	Yes	Membrane
3	WP_000215388.1	N-acetyl mannosaminyl transferase	254	Yes	Cell surface
4	WP_000340131.1	UDP-N-acetylmuramoyl-L-alanyl -D-glutamate--L-lysine ligase	494	Yes	Cytoplasmic
5	WP_000342192.1	Cell division protein FtsQ	439	Yes	Extracellular
6	WP_000358006.1	UDP-N-acetylglucosamine 1 -carboxytransferase 1	421	Yes	Cytoplasmic
7	WP_000391033.1	Cell division protein FtsA	470	Yes	Cytoplasmic
8	WP_000728751.1	Peptidoglycan binding protein	516	Yes	Cell surface
9	WP_000787940.1	Multispecies: Cell division protein FtsW	408	Yes	Membrane
10	WP_000919776.1	Penicillin-binding protein 3	691	Yes	Extracellular
11	WP_000991504.1	PTS alpha-glucoside transporter subunit IIBC	534	Yes	Membrane
12	WP_001123276.1	Tautomerase	61	Yes	Cytoplasmic
13	WP_001125540.1	Multispecies: 50S ribosomal protein L35	66	Yes	Extracellular
14	WP_001274017.1	Multispecies: 30S ribosomal protein S20	83	Yes	Extracellular

Primary[a]: Proteins are essential, non-human homolog, and virulent. Secondary[b]: Localization of proteins

Table 2: Drug targets based on all three filtrations

Protein name	Filtration		Pathway	Choke point	Druggability	PDB
	Primary[a]	Secondary[b]				
UDP-N-acetylmuramoyl-L-alanyl -D-glutamate--L-lysine ligase	Yes	Cytoplasmic	Peptidoglycan synthesis	Yes	Druggable	4C13
Cell division protein FtsA	Yes	Cytoplasmic	Cell cycle	No	Novel	3WQT
Penicillin-binding protein 3	Yes	Extracellular	Peptidoglycan synthesis	No	Druggable	3VSK
Tautomerase	Yes	Cytoplasmic	Xylene, Benzoate, Dioxin, and aromatic degradation	No	Druggable	2X4K

Primary[a]: Proteins are essential, non-human homolog, and virulent. Secondary[b]: Localization of proteins

Table 3: Domains of peptidoglycan binding protein

Peptidoglycan binding protein	Domain ID	Domain or repeat name	Amino acid residues	Biological process
	IPR005877	YSIRK Gram-positive signal peptide	2-36	-
	IPR003132	Protein A, Ig-binding domain	37-327	Pathogenesis and IgG binding
	IPR018392	LysM domain	422-466	-
	IPR019931	LPXTG cell wall anchor domain	475-516	-
	IPR005038	Octapeptide repeat	326-421	Immunoglobulin binding

Table 4: Epitopes predicted in peptidoglycan binding protein

Rank	Epitope	Location	Score
1	NLNEEQRNG	174-182	1.000
2	LKDDPSQSAN	187-196	0.878

only peptidoglycan binding protein constitutes of immunoglobulin G (IgG) binding domain. Peptidoglycan binding protein is characterized with four different types of domains and one octapeptide repeat (Table 3) based on InterProScan analysis. Of these five domains, major part of the protein residues includes IgG binding domain ranging from 38 to 327 residues. Further, epitopes are also predicted (Table 4) by SVMTriP as they can bind to the host immune antibodies. A total of nine epitopes were predicted, of which first two epitope sequences have a high score and the residues fall in the IgG domain. This result indicates that peptidoglycan may be probable vaccine candidate for *S. aureus* infections. Finally, the broad spectrum analysis and non-human gut floral analysis was also carried out, and the results indicated that these sequences were not much conserved with other pathogens nor with any human gut flora. As these are non-human gut floral proteins, they can be considered as best therapeutic candidates.

In our study, we could identify the putative drug targets and vaccine candidates, which are majorly involved in three main pathways peptidoglycan synthesis, cell cycle, and xenobiotic degradation. The tautomerase protein plays an important role in xenobiotic degradation, but it consists of only 61 amino acid residues which make it unfavorable for further *in silico* studies. The other two pathways and their proteins are briefly discussed.

Peptidoglycan synthesis

The cytoplasm of bacteria being hypertonic to its surroundings and to resist from osmotic stress, a chain of identical molecules with semi-rigid nature called peptidoglycan layer is synthesized. The peptidoglycan layer is made up alternating of amino sugars, N-acetylglucosamine, and N-acetylmuramic acid (NAM). These layers of NAM and NAG are interconnected by the peptide formed from NAM [49]. As the peptidoglycan layer protects the cell from stress, turgor pressure, and lysis, its integrity is, therefore, very much essential in the survival of bacteria [50]. The peptidoglycan layer is synthesized by series of enzymes and proteins which forms the best drug targets. According to Reed *et al*., the peptidoglycan can be synthesized by a minimum number of genes, but it loses it pathogenicity and resistance to antibiotics [50]. In our study, we have identified two targets which play key role in peptidoglycan synthesis, *viz.,* UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase (MurE) and penicillin-binding protein 3. MurE enzyme play an important role adding the L-lysine amino acid at the third position of the stem peptide, the lowered activity in *in vivo* of MurE, resulted in accumulation of MurNac and methicillin resistance was reduced [51], makes it the most favorable drug target. In the present study, we have observed some of the interesting features of MurE enzyme like it is essential for survival of the pathogen, it is non-human homolog, bearing virulent character, and plays an important role of adding lysine molecule to third position of stem peptide in the peptidoglycan pathway, which cannot be done by any other alternate enzymes. All these characteristic features make it one of the suitable drug targets to develop a novel therapeutics against staph infections.

Cell cycle

The series of steps that occur in a cell, for its division and replication (duplication), resulting into two daughter cells is known as cell cycle or cell division. In the cell division process, about 20 proteins form a multiprotein complex, which is known as divisome [52]. These proteins are assembled into the Z-ring structure by a divisome protein FtsZ. This ring structure helps the daughter cells to separate [53]. This structure is anchored to the cytoplasmic membrane by FtsA protein via C-terminal membrane-targeting sequence [54]. FtsA possesses ATPase activity, as it belongs to the actin/MreB protein family [55]. In anticancer drug discovery, the major targets are cytokinesis or cell division proteins of eukaryotes. The drug resistance has also led the path for targeting the prokaryotic cell division proteins as antimicrobials. Some studies show that FtsZ can be the best target in drug-resistant organisms [56]. Here, we have identified cell division protein FtsA as the target protein which anchors the FtsZ ring complex. FtsA, showing ATPase activity, can be one of the attractive and best targets as there are many inhibitors that can act on ATPase [57].

CONCLUSION

The availability of complete proteome of different strains of *S. aureus* and by taking the advantage of current computational technologies, we have carried out the study. By employing the strategic, systematic *in silico* filtration process, the study reports common putative therapeutic candidates. The proteins filtered from the first phase, which satisfies the criteria of essentiality, non-human homolog, and virulence were the probable therapeutic candidates. The proteins that are contributed to be involved in pathways, chokepoints, having PDB structures, and mainly localized in the cytoplasm were characterized as the drug targets. Whereas the proteins that are localized on the cell surface having antigenic property, ≤3 transmembranes, a domain with epitope that can bind host immunoglobulin were characterized as vaccine candidates. By this approach, 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, whereas the best common vaccine candidate includes peptidoglycan binding protein. MurE was found to be druggable target and FtsA to be a novel drug target. Further studies can define the probable compounds inhibiting the target molecules, which can be further used as alternative treatments. The systematic filtration process can further be employed on other pathogens of clinical interest, to identify rapidly and with ease the alternative therapeutic candidates.

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Table S1: Common essential and non-human homolog proteins in all strains

Serial number	Strain	Protein ID	Protein Name	Length
1	In all the 44 strains of *Staphylococcus aureus*	WP_000050762.1	PTS ascorbate transporter subunit IIA	147
2		WP_000145499.1	Multispecies: Hypothetical protein	339
3		WP_000184370.1	Multispecies: Transglycosylase	301
4		WP_000244865.1	Multispecies: Septation ring formation regulator EzrA	564
5		WP_000340131.1	UDP-N-acetylmuramoyl-L-alanyl -D-glutamate--L-lysine ligase	494
6		WP_000342192.1	Cell division protein FtsQ	439
7		WP_000358006.1	UDP-N-acetylglucosamine 1-carboxytransferase 1	421
8		WP_000391033.1	Cell division protein FtsA	470
9		WP_000409682.1	Multispecies: Ribonuclease P protein component	115
10		WP_000533493.1	Helicase DnaB	466
11		WP_000725225.1	Multispecies: Hypothetical protein	255
12		WP_000787940.1	Multispecies: Cell division protein FtsW	408
13		WP_000803157.1	Nuclease SbcCD subunit C	1009
14		WP_000834090.1	Hypothetical protein	476
15		WP_000876756.1	Multispecies: Transcriptional regulator	250
16		WP_000919776.1	Penicillin-binding protein 3	691
17		WP_000991504.1	PTS alpha-glucoside transporter subunit IIBC	534
18		WP_001123276.1	Tautomerase	61
19		WP_001125540.1	Multispecies: 50S ribosomal protein L35	66
20		WP_001125619.1	N-acetylmuramoyl-L-alanine amidase	619
21		WP_001274017.1	Multispecies: 30S ribosomal protein S20	83

S. aureus: Staphylococcus aureus

Table S2: Common essential and non-human homolog proteins in some of the strains

Strain	Protein ID	Protein name	Length
BSAR706/8987	CPQ78240.1	AmrA	476
BSAR863/9061	CPJ34910.1	AmrA	476
M0408	WP_000145497.1	Chitinase	339
SA3-LAU	WP_029549721.1	Chitinase	339
930918-3	WP_001077826.1	Cobalt ABC transporter permease	277
M21126	WP_031787615.1	Cobalt ABC transporter permease	277
RF122	WP_000046022.1	Delta-hemolysin	26
21262	WP_000046022.1	Delta-hemolysin	26
21269	WP_000046023.1	Delta-hemolysin	26
LGA251	WP_000046022.1	Delta-hemolysin	26
ED1333	WP_000046023.1	Delta-hemolysin	26
M0406	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
M1216	WP_000473651.1	Glucose-specific phosphotransferase enzyme IIA component	166
MR1	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
VRS2	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
21310	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
21334	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
CM05	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
JH9	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
N315	WP_000473653.1	Glucose-specific phosphotransferase enzyme IIA component	166
M0408	WP_000736790.1	Glycyl-glycine endopeptidase LytM	316
SF1585	WP_000736790.1	Glycyl-glycine endopeptidase LytM	316
TW20	WP_000736790.1	Glycyl-glycine endopeptidase LytM	316
21202	WP_000736800.1	Glycyl-glycine endopeptidase LytM	316
MRSA252	WP_000736790.1	Glycyl-glycine endopeptidase LytM	316
S2398	WP_000736790.1	Glycyl-glycine endopeptidase LytM	316
21262	WP_000271552.1	Holliday junction DNA helicase RuvA	200
M21126	WP_000271553.1	Multispecies: Holliday junction DNA helicase RuvA	200
SA3-LAU	WP_029549861.1	Polysaccharide extrusion protein	476
SA083	WP_043044852.1	Polysaccharide extrusion protein	476

(Contd...)

Table S2: *(Continued)*

Strain	Protein ID	Protein name	Length
RF122	WP_000505013.1	Protein GlcT	283
21262	WP_000505013.1	Protein GlcT	283
21269	WP_000505012.1	Protein glct	283
LGA251	WP_000505013.1	Protein glct	283
ED1333	WP_000505013.1	Protein glct	283
21262	WP_001140868.1	Pyrophosphatase	309
JKD6159	WP_001140868.1	Pyrophosphatase	309
N315	WP_001283057.1	RNA polymerase sigma factor SigA	368
M0406	WP_001041111.1	RNA polymerase sigma factor SigB	256
MR1	WP_001041111.1	RNA polymerase sigma factor SigB	256
VRS2	WP_001041111.1	RNA polymerase sigma factor SigB	256
CM05	WP_001041111.1	RNA polymerase sigma factor SigB	256
JH9	WP_001041111.1	RNA polymerase sigma factor SigB	256
N315	WP_001041111.1	RNA polymerase sigma factor SigB	256
BSAR863/9061	CPI77587.1	Secretory antigen	255
BSAR706/8987	CPQ65358.1	Secretory antigen	255
S. aureus 1	WP_047425989.1	Secretory antigen precursor	168
S. aureus 2	WP_047549686.1	Secretory antigen precursor	168
BSAR863/9061	CPI55316.1	Secretory antigen precursor	269
BSAR706/8987	CPQ38910.1	Secretory antigen precursor	269
A9635	WP_000143415.1	Sensor histidine kinase	295
21252	WP_000143414.4	Sensor histidine kinase	295
21202	WP_000143414.4	Sensor histidine kinase	295
BSAR863/9061	CPI66829.1	*Staphylococcal* accessory regulator A	250
BSAR706/8987	CPQ78533.1	*Staphylococcus* accessory regulator a	250
M21126	WP_031787470.1	Teichoic acid biosynthesis protein b	366
S. aureus 1	WP_047427601.1	Teichoic and biosynthesis protein b	367
M0406	WP_001557393.1	Transposase	30
MR1	WP_001557393.1	Transposase	30
VRS2	WP_001557393.1	Transposase	30
21193	WP_001557393.1	Transposase	30
CM05	WP_001557393.1	Transposase	30
JH9	WP_001557393.1	Transposase	30
N315	WP_001557393.1	Transposase	30
BSAR863/9061	CPI58700.1	Uroporphyrin-III C-methyltransferase	118
BSAR706/8987	CPQ40681.1	Uroporphyrin-III C-methyltransferase	118

S. aureus: Staphylococcus aureus

Table S3: Common essential and non-human homolog proteins in specific strains

Strain	Protein ID	Protein name	Length
BSAR706/8987	CPQ99501.1	3-oxoacyl-ACP syntase	69
NCTC8325	YP_498671.1	Accessory regulator-like protein	250
1189-97	WP_050809366.1	ATPase	651
BSAR706/8987	CPQ29832.1	Cell division protein FtsI	691
S. aureus 1	WP_047423896.1	Chromosome partitioning protein ParB	281
BSAR706/8987	CPR09455.1	Cof family hydrolase	46
M21126	WP_031787927.1	Histidine kinase	370
M21126	WP_031786986.1	Homoserine kinase	304
NCTC8325	YP_501337.1	LysM domain-containing protein	255
21202	WP_001140876.1	Manganese-dependent inorganic pyrophosphatase	309
M21126	WP_000584622.1	Multispecies: DNA double-strand break repair rad50 atpase	978
TW20	WP_000135455.1	Phage tail tape measure protein	2757
M0408	WP_001573496.1	Potassium-transporting ATPase A chain 1	438
S2398	WP_033845065.1	Staphyloxanthin biosynthesis protein	297
M1216	WP_006190740.1	Sucrose operon repressor	316
BSAR706/8987	CPR08741.1	Transmembrane component of general energizing module of ECF transporters	43
930918-3	WP_050346508.1	UDP kinase	90

S. aureus: Staphylococcus aureus

Table S4: Possible drug targets involved in different pathways

Protein name	Pathway ID	Pathway	Enzyme (E.C)
PTS ascorbate transporter subunit IIA	KO00053	Ascorbate and aldarate metabolism	2.7.1.69
	KO02060	Phosphotransferase system	
Multispecies: Transglycosylase	KO00550	Peptidoglycan synthesis	2.4.2.48
UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase	KO00550	Peptidoglycan synthesis	6.3.2.7
cell division protein FtsQ	KO04112	Cell cycle	-
UDP-N-acetylglucosamine 1-carboxytransferase 1	KO00520	Amino sugar and nucleotide sugar metabolism	2.5.1.7
	KO00550	Peptidoglycan synthesis	
cell division protein FtsA	KO04112	Cell cycle	-
multispecies: Cell division protein FtsW	KO04112	Cell cycle	-
penicillin-binding protein 3	KO01501	β-lactam resistance	2.4.1.129
	KO00550	Peptidoglycan synthesis	
PTS alpha-glucoside transporter subunit IIBC	KO00010	Glycolysis/glycogenesis	2.7.1.69
	KO02060	Phosphotransferase system	
Tautomerase	KO00622	Xylene degradation	5.3.2.6
	KO00362	Benzoate degradation	
	KO00621	Dioxin degradation	
	KO01220	Degradation of aromatic compounds	
Multispecies: 50S ribosomal protein L35	KO03010	Ribosome	-
Multispecies: 30S ribosomal protein S20	KO03010	Ribosome	-

Table S5: Druggability analysis of identified drug targets

Protein name	Druggability	Druggable against organism	Drug
PTS ascorbate transporter subunit IIA	Novel	-	-
Multispecies: Transglycosylase	Novel	-	-
UDP-N-acetylmuramoyl-L-alanyl-D -glutamate--L-lysine ligase	Druggable	*Escherichia coli* (strain K12)	Uridine-5'-diphosphate-N-Acetylmuramoyl -L-alanine-D-glutamate[E]; 2,6-diaminopimelic acid[E]; Lysine Nz-carboxylic acid[E]
Cell division protein FtsQ	Novel	-	-
UDP-N-acetylglucosamine 1 -carboxytransferase 1	Druggable	*Escherichia coli* (strain K12)	Fosfomycin[A]
Cell division protein FtsA	Novel	-	-
Multispecies: Cell division protein FtsW	Novel	-	-
-binding protein 3	Druggable	*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4)	Cloxacillin[A]; Cefprozil[A]; faropenem medoxomil[I]
PTS alpha-glucoside transporter subunit IIBC	Novel	-	-
Tautomerase	Druggable	Pseudomonas putida	2-Oxo-3-pentenoic acid[E]
Multispecies: 50S ribosomal protein L35	Novel	-	-
Multispecies: 30S ribosomal protein S20	Druggable	*Thermus thermophilus (strain HB8/ATCC 27634/DSM 579)*	2-methylthio-n6-isopentenyl -adenosine-5'-monophosphate[E]

[A]Approved [I]Investigating [E]Experimental. *E. coli: Escherichia coli, S. pneumoniae: Streptococcus pneumoniae , P. putida: Pseudomonas putida, T. thermophilus: Thermus thermophilus*