S. aureus, a Gram-positive, facultative anaerobe, commensal, and has been evolved [17]. The evolvement of different strains MRSA or nosocomial MRSA and community acquired-MRSA strains have been evolved in two forms of infections hospital acquired- Staphylococcal gene acquired from distant species. This methicillin-resistant With a period, these bacteria have acquired methicillin resistance oxacillin, which mainly act on penicillin-binding proteins. β-lactam antibiotics such as methicillin, penicillin, cephalosporins, and factors leading to death. These infections are most commonly treated by numerous diseases, ranging from minor skin infections to severe and lethal humans [4]. The major transmission of such research due to lack of profits [18]. 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].

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. 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 in 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 different strains of *S. aureus* and 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 check point 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 proteins that are localized on cell surface were analyzed, and then druggability or novelty of the target was done by BLASTP option from Human BLASTP [21], PSORT [24]. Similarly, these proteins 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 check points 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 each 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 (S1-S3)). The virulence property for the common proteins from all

![Fig. 1: Distribution of pandemics caused by *Staphylococcus aureus* in past decades](image-url)
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. \[\text{PTS alpha-glucoside transporter subunit IIIBC}\]
   \[\text{Protein EIIB N(pj)-phospho-L-histidine/cysteine + sugar = protein EIIB + sugar phosphate.}\]

b. \[\text{UDP-N-acetylglucosamine 1-carboxytransferase 1}\]
   \[\text{Phosphoenolpyruvate + UDP-N-acetyl-alpha-D-glucosamine = phosphate + UDP-N-acetyl-3-O-(1-carboxyvinyl)-alpha-D-glucosamine.}\]

c. \[\text{UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase}\]
   \[\text{ATP + UDP-N-acetyl-alpha-D-muramoyl-L-alanyl-D-glutamate + L-lysine = ADP + phosphate + UDP-N-acetyl-alpha-D-muramoyl-L-alanyl-gamma-D-glutamyl-L-lysine.}\]

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 muramoylsaminly transferase, whereas peptidoglycan binding protein constitutes only one transmembrane helix ranging between 12 and 34 residues. The InterProScan have shown that the targets have crystal structures. The 3-dimensional crystal structures of proteins define its biological activity and also define the topology 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).

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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-acetylmuramic acid, and 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 its 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 mexitillin 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 alternative 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 FtsAs 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, checkpoints, 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 unfavorable 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-acetylmuramyl-L-alanyl-D-glutamate-L-lysine ligase | 494    |
| 6              |        | WP_000342192.1 | Cell division protein FtsQ | 439    |
| 7              |        | WP_000358006.1 | UDP-N-acetylglycosamine 1-carboxyltransferase 1 | 421    |
| 8              |        | WP_000409682.1 | Cell division protein FtsA | 470    |
| 9              |        | WP_000342192.1 | 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 | Nucleoside SbcCD subunit G | 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 | Multispecies: 30S ribosomal protein S20 | 83     |

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_000460221.1 | Delta-hemolysin | 26     |
| 21262          | WP_000460221.1 | Delta-hemolysin | 26     |
| 21269          | WP_000460221.1 | Delta-hemolysin | 26     |
| LGA251         | WP_000460221.1 | Delta-hemolysin | 26     |
| EDI333         | WP_000460221.1 | Delta-hemolysin | 26     |
| M0406          | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| M1216          | WP_004736511.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| MR1            | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| VRS2           | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| 21310          | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| 21334          | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| CM05           | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| JH9            | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| N315           | WP_004736531.1 | Glucose-specific phosphotransferase enzyme IIA component | 166    |
| M0408          | WP_000736790.1 | Glycyl-glycine endopeptidase IytM | 316    |
| SF1585         | WP_000736790.1 | Glycyl-glycine endopeptidase IytM | 316    |
| TW20           | WP_000736790.1 | Glycyl-glycine endopeptidase IytM | 316    |
| 21202          | WP_000736800.1 | Glycyl-glycine endopeptidase IytM | 316    |
| MRSA252        | WP_000736790.1 | Glycyl-glycine endopeptidase IytM | 316    |
| S2398          | WP_000736790.1 | Glycyl-glycine endopeptidase IytM | 316    |
| 21262          | WP_000271552.1 | Holliday junction DNA helicase RuVA | 200    |
| M21126         | WP_000271552.1 | Holliday junction DNA helicase RuVA | 200    |
| SA3-LAU        | WP_029549861.1 | Polysaccharide extrusion protein | 476    |
| SA083          | WP_043048521.1 | 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    |
| JK6615 | WP_001140868.1 | Pyrophosphatase                                   | 309    |
| N315   | WP_000283057.1 | RNA polymerase sigma factor SigA                  | 368    |
| M0406  | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| MR1    | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| VRS2   | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| CM05   | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| JH9    | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| N315   | WP_000140111.1 | RNA polymerase sigma factor SigB                  | 256    |
| BSAR863/9061 | CPQ77587.1 | Secretory antigen                                | 255    |
| BSAR706/8987 | CPQ65358.1 | Secretory antigen                                | 255    |
| S. aureus 1 | WP_0047425989.1 | Secretory antigen precursor                      | 168    |
| S. aureus 2 | WP_004754968.1 | Secretory antigen precursor                      | 168    |
| BSAR863/9061 | CPQ53316.1 | 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 | CPQ68629.1 | Staphylococcal accessory regulator A             | 250    |
| BSAR706/8987 | CPQ78533.1 | Staphylococcal accessory regulator a             | 250    |
| M21126 | WP_001178470.1 | Teichoic acid biosynthesis protein b              | 366    |
| S. aureus 1 | WP_004742760.1 | Teichoic acid biosynthesis protein b              | 367    |
| M0406  | WP_000155393.1 | Transposase                                      | 30     |
| MR1    | WP_000155393.1 | Transposase                                      | 30     |
| VRS2   | WP_000155393.1 | Transposase                                      | 30     |
| 21193  | WP_000155393.1 | Transposase                                      | 30     |
| CM05   | WP_000155393.1 | Transposase                                      | 30     |
| JH9    | WP_000155393.1 | Transposase                                      | 30     |
| N315   | WP_000155393.1 | Transposase                                      | 30     |
| BSAR863/9061 | CPQ58700.1 | Uroporphyrin-III C-methyltransferase             | 118    |
| BSAR706/8987 | CPQ40681.1 | Uroporphyrin-III C-methyltransferase             | 118    |

* S. aureus: Staphylococcus aureus

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### 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     |
| NCT03325  | EP_498671.1  | Accessory regulator-like protein                 | 250    |
| 1189-97   | WP_005080936.1 | ATPase                                         | 651    |
| BSAR706/8987 | CPQ29832.1 | Cell division protein Fts1                      | 691    |
| S. aureus 1 | WP_004742389.1 | Chromosome partitioning protein ParB            | 281    |
| BSAR706/8987 | CPQ09455.1 | Cof family hydrolyase                           | 46     |
| M21126   | WP_001789729.1 | Histidine kinase                               | 370    |
| M21126   | WP_001786986.1 | Homoserine kinase                              | 304    |
| NCT03325  | WP_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_001573946.1 | Potassium-transporting ATPase A chain 1         | 438    |
| S2398    | WP_003384506.5 | Staphyloxanthin biosynthesis protein             | 297    |
| M1216    | WP_006190740.1 | Sucrose operon repressor                        | 316    |
| BSAR706/8987 | CPQ8741.1 | Transmembrane component of general energizing module of ECF transporters | 43     |
| 930918-3  | WP_050346508.1 | UDP kinase                                      | 90     |

* S. aureus: Staphylococcus aureus
Table S5: Druggability analysis of identified drug targets

| Protein name | Druggability | Drug | Pathway ID | Pathway | Enzyme (E.C) |
|--------------|--------------|------|------------|---------|--------------|
| PTS ascorbate transporter subunit IIA | Novel | - | K000053 | Ascorbate and aldarate metabolism | 2.7.1.69 |
| Multispecies: Transglycosylase | Novel | - | K000055 | Peptidoglycan synthesis | 2.4.2.48 |
| UDP-N-acetylglucosamine 1-carboxyltransferase 1 | Druggable | Escherichia coli (strain K12) | K000555 | Peptidoglycan synthesis | 6.3.2.7 |
| Cell division protein PtsQ | K004112 | Cell cycle | - | Peptidoglycan synthesis | 2.5.1.7 |
| Cell division protein PtsA | K004112 | Cell cycle | - | - | 2.4.1.129 |
| Multispecies: Cell division protein PtsW | K001501 | β-lactam resistance | - | - | 2.4.1.129 |
| Multispecies: Ribosomal protein L35 | K000010 | Glycolysis/glycogenesis | - | - |
| Multispecies: Ribosomal protein S20 | K000010 | Ribosome | - | - |

Table S5: Possible drug targets involved in different pathways

| Protein name | Pathway ID | Pathway | Enzyme (E.C) |
|--------------|------------|---------|--------------|
| PTS ascorbate transporter subunit IIA | K000053 | Ascorbate and aldarate metabolism | 2.7.1.69 |
| Multispecies: Transglycosylase | K000055 | Peptidoglycan synthesis | 2.4.2.48 |
| UDP-N-acetylglucosamine 1-carboxyltransferase 1 | K000555 | Peptidoglycan synthesis | 6.3.2.7 |
| Cell division protein PtsQ | K004112 | Cell cycle | - |
| Multispecies: Cell division protein PtsW | K001501 | β-lactam resistance | - |
| Multispecies: Ribosomal protein L35 | K000010 | Glycolysis/glycogenesis | 2.7.1.69 |
| Multispecies: Ribosomal protein S20 | K000010 | Ribosome | - |

**Approved**
**Investigating**
**Experimental**

E. coli: Escherichia coli, S. pneumoniae: Streptococcus pneumoniae, P. putida: Pseudomonas putida, T. thermophilus: Thermus thermophilus