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Identification and characterization of potential membrane-bound molecular drug targets of methicillin-resistant *Staphylococcus aureus* using *in silico* approaches

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Aim. To identify novel putative drug targets of methicillin-resistant S. aureus (MRSA) through subtractive proteome analysis. **Methods.** Identification of non-homologous proteins in the human proteome, search of MRSA essential genes and evaluation of drug target novelty were performed using a protein BLAST server. Unique metabolic pathways identification was carried out using data and tools from KEGG (Kyoto Encyclopedia of Genes and Genomes). Prediction of sub-cellular proteins localization was performed using combination of PSORT v. 3.0.2, CELLO v. 2.5, iLoc-Gpos, and Pred-Lipo tools. Homology modeling was performed using SWISS-MODEL, Phyre2, I-TASSER web-servers and the MODELLER software. **Results.** Proteomes of six annotated methicillin-resistant strains : MRSA ATCC BAA-1680, H-EMRSA-15, LA MRSA ST398, MRSA 252, MRSA ST772, UTSW MRSA 55 were initially analyzed. The proteome analysis of the MRSA strains in several consequent steps allowed to identify two molecular targets: diadenylate cyclase and D-alanyl-lipoteichoic acid biosynthesis (DltB) protein which meet the requirements of being essential, membrane-bound, non-homologous to human proteome, involved in unique metabolic pathways and new in terms of not having approved drugs. Using the homology modeling approach, we have built three-dimensional structures of these proteins and predicted their ligand-binding sites. **Conclusions.** We used classical bioinformatics approaches to identify two molecular targets of MRSA :diadenylate cyclase and DltB which can be used for further rational drug design in order to find novel therapeutic agents for treatment of multidrug resistant staphylococcal infection.

Keywords: molecular drug targets; methicillin-resistant *Staphylococcus aureus*; MRSA; subtractive proteome analysis.

Introduction

Staphylococcus aureus is the leading cause of hospital-acquired infections, which range from mild skin and soft tissue infections to more severe diseases, such as endocarditis, bacteremia, sepsis, and osteomyelitis [1]. *S. aureus* belongs to ESKAPE pathogens that are difficult to treat due to increasing multidrug resistance. Penicillin was effective against staphylococcal infections until strains evolved the mechanism to hydrolyse the β-lactam ring of antibiotic. Staphylococcal resistance to penicillin is mediated by penicillinase. Today, in clinical practice methicillin – chemically modified penicillin which cannot be hydrolysed by penicillinase – is widely used for treatment of staphylococcal infections. At present, methicillin-resistant *S. aureus* (MRSA) is one of the most feared strains of *S. aureus* which have become resistant to most of β-lactam antibiotics. For this reason, vancomycin is commonly used to combat MRSA. Recently, the vancomycin-resistant *S. aureus* strains have also emerged. Noteworthy, none drug to date has shown superiority to vancomycin in the treatment of MRSA infections with the possible exception of linezolid in hospital-acquired pneumonia [2]. Therefore, the development of novel antibiotics for the treatment of staphylococcal infections is of great interest. The ability of bacteria to rapidly acquire drug resistance requires the selection of novel proper molecular targets. Today, a number of proteins are considered as promising drug targets for the development of antibiotics to treat staphylococcal infections. After extensive review of the literature for the last three years, the following proteins were considered as potential therapeutic drug targets for the development of antistaphylococcal agents: bacterial enoyl reductase (FabI) [3,4], transglycosylase (TGase) [5,6], sortase A [7-13], diapophytoene desaturase (CrtN) [14-17], type II topoisomerase [18-21], topoisomerase IV [22-27], filamentous temperature-sensitive protein Z (FtsZ) [28-30], UDP-N-acetylenolpyruvylglucosamine reductase (MurB) [31], lipoteichoic acid synthase (LtaS) [32], biotin protein ligase [33,34], peptide deformylase [35], Ser/Thr protein kinase STK1 [36], pentaerythritol tetranitrate reductase [37], peptide deformylase (PDF) [38, 39], NorA efflux pump [40-46], poly-beta-1,6-N-acetyl-D-glucosamine synthase (IcaA) [47], dihydrofolate reductase (DHFR) [48], phenylalanine tRNA synthetase (PheRS) [49],4-dihydroxy-2-naphthoate prenyltransferase (MenA) [50], SecA ATPase [51], biotin protein ligase (BPL) [52], UDP-MurNAc-pentapeptide (MurF), uridine monophospate kinase (UMPK) [53], N-Acetylneuraminate lyase [54], 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) [55, 56], caseinolytic protease ClpP [57], multidrug efflux pump LmrS [58], collagen (Cn)-binding protein Can [59], pantothenate kinase [60], undecaprenyl diphosphate synthase [61, 62] and undecaprenyl diphosphate phosphatase [61, 63], MurE [64], bacterial pathway for fatty acid biosynthesis, FASII [65], pyruvate kinase [66, 67], nitric oxide synthase [68], eukaryotic-like Ser/Thr phosphatase Stp1 [69], tyrosyl-tRNA synthetase (TyrRSs) [70], heptaprenyl diphosphate synthase (HepPPS) [71], β-Ketoacyl-ACP synthases (KAS) [72], RNA polymerase [73], UDP-GlcNAc 2-epimerase (MnaA) [74],

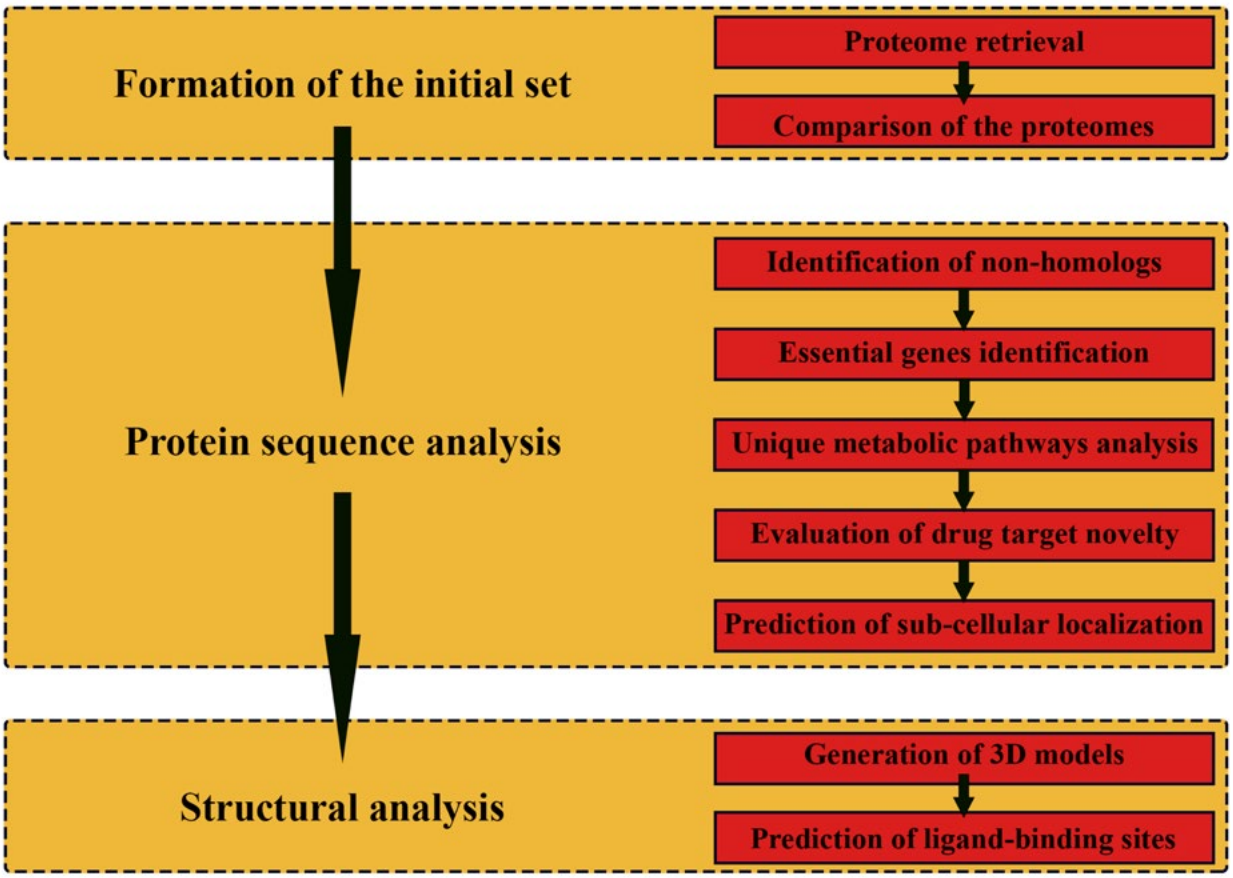


Fig. 1. The workflow of the analysis

ATPase [75], mevalonate diphosphate decarboxylase (MDD) [76], glycosyltransferase [77].

In spite of large amount of proposed molecular targets, there are currently only nine investigational antibiotics against *S. aureus*, including MRSA, undergoing clinical trials but targeting only four proteins, such as DNA gyrase, topoisomerase IV, enoyl-acyl-carrier (ACP) reductase (FabI) and P site at the 50S ribosome subunit of bacteria [78].

Due to fast growth of resistant strains, the identification of unique drug targets amongst the resistant pathogens is very important. A number of methods are currently available to identify potential drug targets. Among them, bioinformatics approaches are the most fast and cost-effective. For example, subtractive genome analysis has been already used to identify putative molecular targets for different pathogenic strains of *Staphylococcus aureus*, such as *Staphylococcus aureus* subsp. *aureus MW2* (CA-MRSA) [79], *Staphylococcus aureus* N315 [80], *Staphylococcus aureus* ST398, *S. aureus* 252 [81, 82], vancomycin-resistant *Staphylococcus aureus* [83]. The aim of this

study is the identification of novel membrane-bound putative molecular targets common for all already sequenced methicillin-resistant strains of *Staphylococcus aureus*.

Methods

The procedure of potential drug targets identification in our work included formation of the initial set, protein sequence analysis, and structural analysis. The workflow is presented in Figure 1.

1. Proteome retrieval of MRSA strains

Complete proteomes of six methicillin-resistant strains of *S. aureus* were downloaded in FASTA format from the NCBI Protein database that contains sequences from GenBank, TPA, RefSeq, PRF, PIR, SwissProt, and PDB on April 10, 2018. Besides the sequences themselves, NCBI also provided general information about the targeted strains genome annotation data.

2. Comparison of the proteomes

In order to form the initial set of proteins for the analysis, [the] NCBI accession numbers (ACs) of the proteins from different strains were compared manually. Only the proteins with common ACs were included into the initial set, the others were not considered, because they were suggested to have some differences between the strains which might have an impact on the potential drug effectiveness.

3. Identification of non- homologous proteins to the human proteome

The representative set was subjected to Protein BLAST against human proteome with the expectation-value cutoff of 10^{-3}. BLOSUM62 was chosen as the scoring matrix for the BLASTP algorithm, the non-redundant protein sequences were taken as the search set database. As a result, we have obtained homologous sequences, with significant similarity to human proteome, and non-homologous sequences, for which no hits with significant similarity were found. The proteins with homology to the human proteome were excluded from the set, thus were not taken into account during further analysis.

4. MRSA essential genes identification

The set of non-homologous proteins was further subjected for alignment against the Database of Essential Genes (DEG) [84, 85]. The current version of this database contains essential genes of two *S. aureus* strains – NCTC 8325 and N315. The sequences were filtered using following settings: BLASTP as the algorithm, BLOSUM62 as the substitution matrix, expectation value cutoff equals to 10^{-5}, minimal score equals to 100. The proteins which met the E-value cutoff and minimal score were considered to be essential for pathogen survival and propagation.

5. Unique metabolic pathways identification

Using the data and tools from KEGG (Kyoto Encyclopedia of Genes and Genomes), the metabolic pathway analysis was carried out to determine unique metabolic pathways of the pathogen. KEGG is an integrated database that contains systems, genomic, chemical and health information allowing biological interpretation of genome sequences and other throughput data [86, 87]. All the essential non-

homologous proteins were analyzed by KEGG Automatic Annotation Server (KAAS). It provides functional annotation of genes or proteins by BLAST or GHOST comparisons against manually curated KEGG databases [88]. In this study we used BLASTP as the algorithm for such comparison. As a result we obtained a KO assignment list, on the basis of which the manual selection of proteins involved in unique metabolic pathways was performed.

6. Evaluation of drug target novelty

To separate already-known drug targets from novel ones, protein BLAST against DrugBank database was performed. The latest version of DrugBank (5.1.0) contains 11 143 drug entries, including 2 555 approved small molecule drugs, 965 approved biotech drugs, 121 nutraceuticals, and over 5 145 experimental drugs. 5121 non-redundant protein sequences are linked to these entries [89]. This allowed us to exclude the proteins that have proper ligands from further analysis.

7. Prediction of sub-cellular localization of proteins

The important question to be addressed when choosing a target for further drug development is the localization of that particular target inside a cell. Compartment localization determines the methods for protein extraction and purification, which makes upcoming investigational steps easier or harder. In order to predict the sub-cellular localization of druggable non-homologous essential proteins a combination of tools such as PSORT v. 3.0.2 [90], CELLO v. 2.5 [91], iLoc-Gpos [92], and Pred-Lipo [93] was used. The localization was assigned to a protein only in the case when all four tools obtained the same results.

8. Homology modeling of proteins

Three dimensional models for the resulting protein set were generated on the basis of homology modeling by web-servers SWISS-MODEL (https://swissmodel.expasy.org/interactive) [94-99], Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [100, 101] and I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) [102-105] with default parameters and Modeller 9.17 using basic modeling mode (https://salilab.org/modeller/tutorial/basic.html) [106-109]. The models were minimized with GROMACS software [110-112] using steepest descent algorithm (1000 steps of minimization). The accuracy of this modeling was validated using MolProbity web-server [113]. The structures for template proteins were retrieved from the Protein Data Bank. In cases, when the performing of homology modeling was impossible, the 3D-structure was built *ab initio* using I-TASSER web-server. The modeled structures for the target proteins were analyzed with ProBiS Tools [114] in order to predict the binding sites of the proteins.

Results and Discussion

The objective of this study was to identify the novel putative drug targets of methicillin-resistant *S. aureus* (MRSA) through subtractive genomic analysis. The combination of subtractive genomic analysis and comparative genomics/proteomics is a powerful method for identification of unique sequences with certain metabolic functions.

Noteworthy, not all methicillin-resistant strains of *S. aureus* have either their genomes

sequenced completely or proteomes annotated. Therefore, in this study we have compared annotated proteomes of already sequenced methicillin-resistant strains, namely MRSA ATCC BAA-1680 [115], H-EMRSA-15 [116], LA MRSA ST398 [117], MRSA 252 [118], ST772 MRSA [119], and UTSW MRSA 55 [120], in order to identify common sequences.

The total number of proteins for each strain, which have been retrieved from NCBI Protein database, is indicated in Table 1.

Following the procedure indicated in Fig. 1, we analyzed the proteome of MRSA in several steps; the results are given in Table 2.

The comparative analysis of protein accession numbers showed that only 326 sequences are common for the abovementioned MRSA strains, the corresponding accession numbers and definitions are given in Supplementary table 1. The resulting set does not exhaust the pool of common proteins due to possible inconsistencies between the accession numbers originated from different sources but can still be considered adequate for the search of common molecular targets.

Table 1. **Total number of proteins corresponding to strains during analysis**

Strain	Isolate (if any reference available)	Total number of proteins
MRSA ATCC BAA-1680	25b	2874
	26b	2872
	27b	2873
	29b	2873
	31b	2873
H-EMRSA-15	-	2775
LA MRSA ST398	-	2767
MRSA 252	-	2819
MRSA ST772	-	2806
UTSW MRSA 55	-	2976

Table 2. **Subtractive analysis results for MRSA**

Analytical step of the analysis	Total number of proteins
Representative set of MRSA proteins	326
Non-homologous proteins	172
Essential proteins	45
Proteins after KEGG analysis	28
Potentially novel drug targets	22
Membrane-bound proteins	2

The existence of homologous proteins between bacteria and human is believed to have emerged in course of evolution [121-123]. A number of studies assume a "similarity hypothesis" that states homology as an evolutionary adaptation of pathogens to prevent being recognized by host's immune system [124, 125]. The selection of particular homologous pairs as potential drug targets against MRSA might lead to cross-reactivity in human hosts. That is the reason why in the next step the sequences from the representative set were subjected to Protein BLAST against the whole human genome with a threshold E-value of 10^{-3}. The proteins with significant similarity were excluded from further analysis to prevent cross-reactions between human and pathogen during pharmaceutical treatment. This step reduced the number of sequences in the representative set to 172.

We determined the essentiality of the non-homologous proteins of methicillin-resistant *S. aureus* based on bioinformatics prediction through homology search in DEG against known essential genes identified by an antisense RNA technique of two *S. aureus* strains, NCTC 8325 and N315. This approach has a crucial useful feature. Essential genes of an organism constitute the minimal set of genes required for a living cell in given growth con-

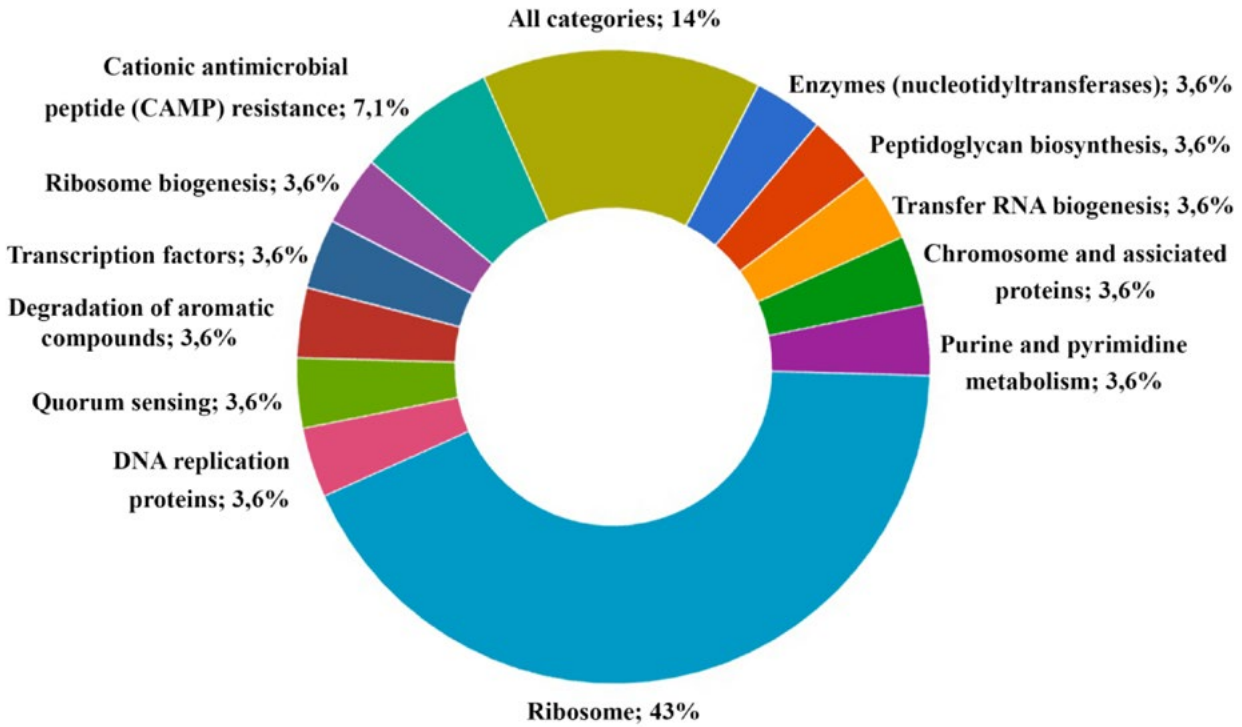


Fig. 2. Orthology groups of analyzed proteins

ditions. Thus, the proteins, coded by corresponding essential genes, have higher druggability potential. Protein BLAST against DEG revealed that 45 sequences from our set had homologs with sequences from DEG. Noteworthy, a certain distribution of the amount of homologs between our set and DEG was observed, e.g., one sequence had 4 homologs in DEG, 11 sequences had 2 homologs each, and the rest of the set had just one corresponding homolog. Supplementary table 2 contains the results obtained during performing this step.

KAAS was used to determine whether the resulted outcome from the DEG step was involved in essential metabolic pathways. More importantly, the analysis with KAAS enabled us to exclude non-homologous essential proteins potentially having the enzymatic activity for reactions of human metabolism or a similar function regarding genetic information processing or signaling and cellular processes. The comparison was carried out between *S. aureus* and human metabolic networks and revealed that 28 sequences out of 45 had a significant similarity to *S. aureus* enzymes and at the same time had none to human. After the manual revision of KO numbers all sequences were classified according to their orthology groups, the distribution of the analyzed proteins throughout KEGG metabolic networks is represented in Figure 2.

Since the objective of our study was to identify the novel putative drug targets we

have evaluated the druggability of the predicted in previous steps protein set by Protein BLAST analysis against DrugBank database (DBD) v. 5.1.0. DrugBank is a freely available web resource containing detailed drug, drug-target, drug action and drug interaction information about FDA-approved drugs and experimental drugs going through the FDA approval process. The results, as shown in Table 3, indicate that 6 proteins out of the analyzed set interact with certain drugs.

Further, the sub-cellular localization of each protein has been predicted using PSORT v. 3.0.2, CELLO v. 2.5, iLoc-Gpos, and PredLipo. The results are presented in Supplementary table 3. Together, the obtained results provide us with a set of novel putative drug targets of MRSA, including two membrane-bound proteins, namely TIGR00159 family protein (diadenylate cyclase) and WP_000613541.1 (D-alanyl-lipoteichoic acid biosynthesis protein DltB).

Diadenylate cyclase is an essential bacterial enzyme which utilizes two molecules of adenosine triphosphate (ATP) for the synthesis of the important second messenger – cyclic diadenylate monophosphate (c-di-AMP) which has been shown to regulate such processes as virulence, cell wall formation, cell size, ion transport, *etc.* Therefore, diadenylate cyclase is a potential target for the development of novel antibiotics. But only a small amount of low-molecular inhibitors for bacterial diadenylate cyclase has been reported in scientific literature so far. Recently, it was shown that several polyphenols inhibit *Bacillus subtilis* diadenylate cyclase [126]. Also, it was found that suramin, known antiparasitic drug is a potent inhibitor of diadenylate cyclase [127]. For the best of our knowledge, none small molecular inhibitor for *S. aureus* diadenylate cyclase has been reported.

DltB is a multi-membrane-spanning protein required for D-alanylation of teichoic acids which is important for the cell wall synthesis. Recently, Pasquina *et al.* [128] using the synthetic lethal approach have identified one compound that inhibits DltB *S. aureus*. It has been found that this inhibitor sensitizes *S. aureus* to several antibiotics and is lethal in combination with a wall teichoic acid inhibitor. Therefore, DltB can be considered as an important antibiotic target as well.

We have generated 3D models for diadenylate cyclase and D-alanyl-lipoteichoic acid biosynthesis protein DltB of *S. aureus*, which can be used for

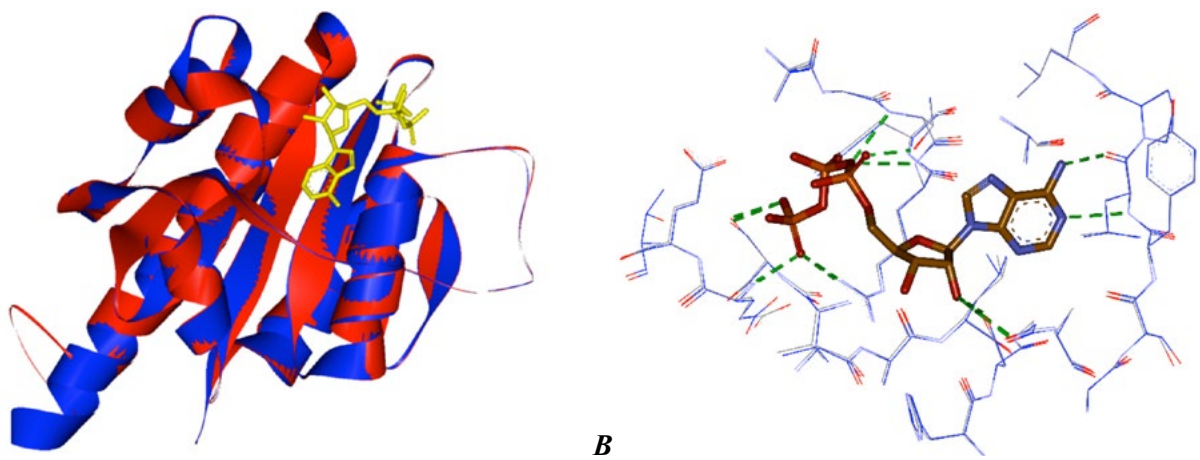
further structure-based drug design. In order to identify template proteins for homology modeling of diadenylate cyclase and DltB

Table 3. **The proteins which interact with the drugs accordingly to DrugBank data**

NCBI Accession Number	The drug	Drug group
WP_000290472.1	Troleandomycin	Approved
WP_001123276.1	2-Oxo-3-Pentenoic Acid	Experimental
WP_001274017.1	**2-methylthio-N6-isopentenyl-adenosine-5**'-monophosphate	Experimental
WP_001549197.1	N-Formylmethionine	Experimental
WP_000692521.1	Flavin mononucleotide	Approved, investigational
WP_000562498.1	Cladribine**,** Gallium nitrate	Approved, investigational

protein we have performed alignment of these sequences against Protein Data Bank (PDB) proteins using BLAST. It has been revealed that the most homologous protein for diadenylate cyclase *S. aureus* is diadenylate cyclase *Listeria monocytogenes* (PDB accession code: 4RV7) [129]. We have built four homology models of diadenylate cyclase of *S. aureus* based on crystal structure of diadenylate cyclase *L. monocytogenes* using web-servers Swiss-Model, Phyre2, I-TASSER and Modeller software tool. These homology models have been validated with MolProbity web-server. The MolProbity score combines the clashscore, rotamer and Ramachandran evaluations. The value of MolProbity score for [the] Swiss-Model homology model is 1.66, for Phyre2 homology model – 2.42, for I-TASSER homology model – 3.85, for Modeller homology model – 3.49. Therefore, according to the obtained results, the homology model which was generated by Swiss-Model server, possesses the best value of MolProbity score and has been taken for further analysis. This homology model was minimized with GROMACS software using steepest descent algorithm (1000 steps). After minimization the MolProbity score was slightly improved (score value is 1.38).

The superposition of homology model of diadenylate cyclase of *S. aureus* (red colour) with crystal structure of diadenylate cyclase *L. monocytogenes* (blue colour) is represented in Figure 3a. RMSD value of diadenylate cyclase of *S. aureus* with template structure is 0.754493. An ATP molecule was chosen as a ligand for modeling. The resulted superimposed structures were further analyzed to locate the binding site residues of modeled diadenylate cyclase beyond the 7 Å radius of the ligand (Figure 3b). The superposition of active sites of homology model (carbon atoms are labeled by green colour) and template structure (carbon atoms are labeled by white colour)



A ***B***

Fig. 3. The superposition of homology model of diadenylate cyclase *S. aureus* (red colour) with crystal structure of diadenylate cyclase *L. monocytogenes* (blue colour) (A) and superposition of active sites of homology model (carbon atoms are labeled by green colour) and template structure (carbon atoms are labeled by white colour) (B). The ligand is an ATP molecule.

demonstrates that the structures of investigated enzymes are very similar. RMSD value of amino acid residues in the active sites of these enzymes is 0.885616.

Using BLAST we have not identified any homologous protein for DltB, therefore the 3D model should to be generated only *ab initio*. We have built the model of DltB protein *S. aureus* using I-TASSER web-server. Confidence score (C-score) for the best model is -1.29. C-score is a confidence score for estimating the quality of predicted models by I-TASSER. It is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score varies typically from -5 to 2. The MolProbity score for this homology model is 3.63.

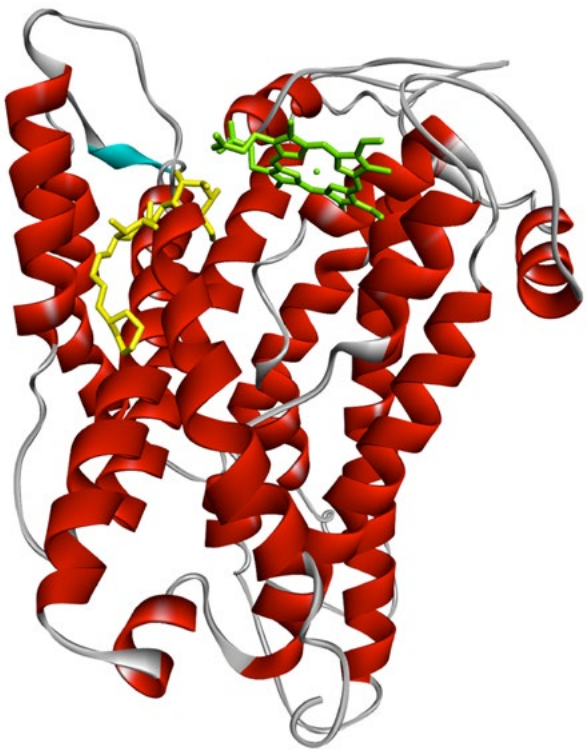


Fig. 4. The homology model of D-alanyl-lipoteichoic acid biosynthesis protein DltB *S. aureus*, obtained with I-TASSER server. The ligands (green heme and yellow CYMAL-4) indicate binding sites for small-molecular compounds.

We tried to optimize I-TASSER homology model with GROMACS using different force fields but there were some problems with atom types. Therefore, we have built the homology model of DltB protein *S. aureus* by Swiss-Model server using as a template 3D model generated with I-TASSER server. This homology model was minimized with GROMACS using steepest descent algorithm (1000 steps). After minimization, the MolProbity score was significantly improved in comparison with the input model. MolProbity score of optimized model is 2.07.

Also, we used ProBis tool to identify the binding sites of modeled DltB protein. Two potential binding sites, which can bind small molecules, have been predicted (see Figure 4). The binding pocket 1 (around green colored heme) is formed by amino acid residues Arg374, Asn377, Asn285, Lys284, Tyr33, Asn34, Val37, Gly35, Thr38, Ile208, Phe46, Ala204, Met41, Lys203, Glu199, Arg198. Pocket 2 (around yellow labeled CYMAL-4) is formed by amino acid residues Met97, Ala382, Phe379, Gly378, Asn377, Gln376, Trp375, Lys108, Arg374.

Conclusion

In this study we used classical bioinformatics approaches to estimate whether there are potential drug targets among methicillin-resistant *Staphylococcus aureus* proteins. Using subtractive genomic analysis we have identified two molecular targets of MRSA – diadenylate cyclase and D-alanyl-lipoteichoic acid biosyn-

thesis protein which can be used for further rational drug design in order to identify novel therapeutic agents for the treatment of multi-drug resistant staphylococcal infection.

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Пошук нових молекулярних мішеней, асоційованих з мембраною, для розробки антибіотиків проти метицилін-резистентного штаму *Staphylococcus aureus in silico*

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Мета. Метою дослідження був пошук нових мішеней для дизайну антибіотиків проти метицилін-резистентного штаму *S. aureus* (MRSA) методами розрахункової протеоміки. **Методи.** Ідентифікація негомологічних білків до людського протеому, визначення генів важливих для виживання MRSA та встановлення новизни знайдених мішеней проводили алгоритмами BLAST. За допомогою утиліт бази даних KEGG ідентифікували унікальні метаболічні шляхи бактерій. Клітинну локалізацію протеїнів передбачали програмами PSORT v. 3.0.2, CELLO v. 2.5, iLoc-Gpos, та Pred-Lipo. Гомологічне моделювання проводили веб-серверами SWISS-MODEL, Phyre2, I-TASSER та MODELLER. **Результати.** Початкову вибірку було сформовано з протеомів шести штамів MRSA: ATCC BAA-1680, H-EMRSA-15, LA MRSA ST398, MRSA 252, MRSA ST772, UTSW MRSA 55. Багатостадійний аналіз вибраних протеомів за допомогою алгоритму BLAST дозволив ідентифікувати дві потенційні молекулярні мішені – диаденілатциклазу та білок DltB, що відповідають заданим вимогам: є важливими для виживання бактерії, є асоційованими з мембранами, не є гомологами людських білків, залучені до унікальних метаболічних шляхів та раніше не досліджувались як терапевтична мішень. Було побудовано просторові структури знайдених протеїнів. **Висновки.** У результаті дослідження методами лінійної біоінформатики запропоновано дві потенційні мішені – диаденілатциклазу та білок DltB, для подальшої розробки антибіотиків проти метицилін-резистентного штаму бактерії *Staphylococcus aureus* методами раціонального пошуку лікарських засобів.

Ключові слова: MRSA, MDR-PA, порівняльна протеоміка, гомологічне моделювання, молекулярні мішені.

Поиск новых мембраносвязанных молекулярных мишеней для разработки антибиотиков против метициллин-резистентного штамма *Staphylococcus aureus in silico*

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Цель. Определить новые мишени для дизайна антибиотиков против метицил-лин-резистентного штамма S. aureus (MRSA) методами расчетной протеомики. **Методы.** Идентификация негомологичных последовательностей с человеческими, определение критических для выживания MRSA белков и определение их новизны как терапевтических мишеней проводилось алгоритмами BLAST. При помощи утилит базы дан-

ных KEGG идентифицировали уникальные метаболические пути бактерии. Клеточную локализацию определяли программным обеспечением PSORT v. 3.0.2, CELLO v. 2.5, iLoc-Gpos и Pred-Lipo. Гоммологическое модели были построены веб-серверами SWISS-MODEL, Phyre2, I-TASSER и программой MODELLER. **Результаты.** Первоначальная выборка была составлена с протеомов шести штаммов MRSA: ATCC BAA-1680, H-EMRSA-15, LA MRSA ST398, MRSA 252, MRSA ST772, UTSW MRSA 55. Многостадийный анализ отобранных протеомов алгоритмом BLAST позволил идентифицировать две потенциальные молекулярные мишени: диаденилатциклазу и белок DltB, соответствующие критериям критичности для выживания, которые не гомологичны к человеческим белкам, ранее не исследовались при разработке антибиотических средств и локализованы в мембране. Также была построена трёхмерная модель найденных мишеней. **Выводы.** В ходе исследования, методами линейной биоинформатики было определено два белка-мишени – диаденилатциклаза и белок DltB, для последующей разработки антибиотических препаратов против метициллин-резистентного штамма Staphylococcus aureus методами рационального поиска лекарственных средств.

Ключевые слова: MRSA, MDR-PA, сравнительная протеомика, гомологическое моделирование, молекулярные мишени.

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