In silico Identification of Drug Targets in Methicillin/Multidrug-Resistant *Staphylococcus aureus*

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***Abstract*— This paper reports an extension of an established bioinformatics approach to a new organism involving more than one strains for comparison. Methicillin/multidrug-resistant *Staphylococcus aureus* causes serious infections in humans and becomes resistant to increasing numbers of antibiotics. Our approach utilizing CD-HIT and BLASP *in silico* tools identified 133 and 134 genes in MRSA 252 strain and MRSA Mu50 strain respectively that are essential to pathogen survival with E-score < 10^{-4} and absent in the human genome with E-score < 10^{-3}. The genes were further classified according to their known or hypothetical or putative functions annotated by NCBI RefSeq and/or Integr8-Inquisitor. A list of central energy metabolic enzymes, which either do not have human homologues or functionally differentiate themselves from their human counterparts through alternative catalytic mechanisms, were considered as promising antibiotic drug targets. We proposed that the development of central energy metabolic inhibitors is a novel approach to avoid antibiotic resistance.**

Keywords

Methicillin/multidrug-Resistant Staphylococcus aureus (MRSA), essential genes, Database of Essential Genes (DEG), drug targets, central metabolism

I. INTRODUCTION

Methicillin/multidrug resistant *Staphylococcus aureus* (MRSA) infections are caused by antibiotic resistant strains of the common bacterium *Staphylococcus aureus* [1]. The beginning signs of MRSA infections are skin infections that resemble pimples, boils or spider bites. In immune-deficient patients, localized skin infections quickly spread through the bloodstream causing vital organ infections and possible death [2]. In a 2007 Centers for Disease Control and Prevention press release, there were about 94,000 cases of MRSA infections, contributing to around 19,000 deaths in the United States in 2005, which implies a mortality rate higher than that caused by HIV [3, 4].

The first MRSA case presented in the United Kingdom in 1961[5]. Shortly after, more variations were identified to be immune to β-lactam antibiotics (including penicillin, methicillin, oxacillin, and cephalosporins [6, 7]). Newly discovered MRSA strains have evolved to survive sulfa drugs, such as tetracyclines, and clindamycin [8]. Glycopeptide antibiotics, such as vancomycin and teicoplanin, considered drugs of "last resort", were used for the treatment of MRSA infections [9, 10]. However, recently discovered MRSA strains showed resistance even to vancomycin and teicoplanin [11, 12]. As of 2007, one variant found was resistant to six major kinds of antibiotics [13].

The current treatment for MRSA infections is still traditional broad-spectrum antibiotics such as lincosamides, sulfa drugs, glycopeptides [14-16], among which linezolids [17] daptomycin [18], Trimethoprim-sulfamethoxazole and MoxifloxacinHCl were considered relatively more effective [19, 20] though MRSA infections have become increasingly difficult to treat [15-17]. Thus, alternative treatments precisely targeting the root cause of MRSA infections needs to be established.

Novel antibiotic development focuses on the following: target screening *vs.* whole organism screening, microarray and/or proteomics [21]; target identification; rational and computer-assisted drug design [22, 23] and combinatorial chemistry *etc.*. The task falls on the shoulder of academia since the pharmaceutical industry has ceased investing in antibiotic discovery owing to high cost, lengthening developing cycles, complexities and low profits along with failure of several recent investments into target-based approaches [24]. In this paper, we report the initial results of anti-MRSA drug development, *i.e.,* a systematic *in silico* approach for the identification of drug targets in two MRSA strains, MRSA 252 and MRSA Mu50 based on the following two criteria: essentiality to pathogen survival and absence from the human genome [25, 26]. The novelty lays in that a special list of enzymes targeting bacterial metabolism was identified, shedding light on a potentially new approach for antibiotic development.

II. METHOD

The objective of this study was to determine potential drug targets for alternative treatment of MRSA infections, to explore hypothetically the functions of the identified targets

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and to shorten the list. We employed a reported *in silico* approach through a systematic and juestified method [27, 28] for the identification of drug targets in two MRSA strains, MRSA 252 and MRSA Mu50. The proteomes of MRSA 252 and Mu50 were retrieved from NCBI gene bank [29]. MRSA genes were purged at 90 % and 60% using CD-HIT [30] to remove paralogues. The resulting sequences were run through the database of essential genes (DEG) [31, 32] at an expectation (E-value) cutoff of 10^{-4}. The database of essential genes includes genes required for basic survival of *Staphylococcus aureus* and other microorganisms according to experimental evidence. The essential genes were subjected to BLASTP against the human genome to exclude any genes that have a significant match (E-value cutoff of 10^{-3} and lower) with human homologs. Genes having BLAST E-scores less than 10^{-3} were considered as having no close relatives in human. Information about the putative gene function was derived from the annotated genome sequence through NCBI RefSeq and Integr8-Inquisitor [33].

III. RESULTS AND DISCUSSION

The goal of this investigation was to determine potential drug targets for alternative treatment of MRSA infections and to classify and to analyze the identified targets. Out of the complete genomes of 13 MRSA strains that were sequenced and deposited in the NCBI gene bank, MRSA 252 and MRSA Mu50 were selected due to the fact that the former is a common strain in USA [34] and UK [35] and the latter, a methicillin and vancomycin resistant strain isolated in Japan [36] is commercially available for future molecular biological study (ATCC). The common method of drug target identification encompasses two steps: the identification of essential genes for bacterial viability [25] and the identification of genes absent in the human genome [26]. The former was performed by adopting the DEG database in our approach because this database compiles a list of all currently available essential genes in more than 10 prokaryotes including *Staphylococcus aureus* [29] and was proved to be more accessible than conventional tools [27, 28]. On the other hand, the availability of the human genome sequence [37, 38] renders the latter step feasible. Following two newly published genomic analysis methods [27, 28], 2656 MRSA 250 and 2697 Mu50 genes were purged at 90 % and 60% using CD-HIT to remove paralogues, respectively. The resulting 2568 MRSA 250 and 2592 Mu50 sequences were run through the database of essential genes (DEG) at an expectation cut-off of 10^{-4}, yielding 499 and 496 essential genes respectively. Those 499 and 496 essential genes identified were subjected to BLASTP against the human genome [37, 38] to exclude any genes that have a significant match (*E*-value cutoff of 10^{-3} and lower) with human homologs. Consensually, 133 MRSA 252 and 134 Mu50 genes respectively were considered as having no close relatives in human. The results are summarized in table 1. Their known or hypothetic or putative functions annotated by NCBI RefSeq Integr8-Inquisitor are listed in table 2.

TABLE 1: GENOMICS ANALYSES OF MRSA 252 AND MRSA MU50 STRAINS.

Genes	MRSA 252	MRSA Mu50
Total number	2656	2697
Duplicates (>60% identical)	88	105
Non-paralogs	2568	2592
Essential genes [cut-off *E*-value < 10^{-4}]	499	496
Essential genes w/o human homologs[cut-off *E*-value < 10^{-3}]	133	134

Among the 133 and 134 essential non-human homologous genes in MRSA 252 and Mu50 strains, respectively, 133 encode proteins that are well conserved between the two strains. Out of this conserved set, 63 are involved in metabolism, 24 participate in the transmission of genetic information, 29 represent transmembrane proteins, 9 are with other functions such as regulation cell division and carrier proteins, *etc.,* and 8 have unknown functions.

Our approach identified 14 genes in cell wall biosynthesis. Other research groups have validated most of these targets [39-41]. Among them, 6 are involved in the elongation of peptidoglycan, in agreement with previous studies [39, 40]. FemA family proteins are currently considered novel anti-staphylococcal targets due to the fact that they are involved in cell wall biosynthesis and expression of a methicillin resistance gene [41]. They are found to be essential in both MRSA 252 (NCBI Gene Accession#: 49484627 and 49483567) and Mu50 (NCBI Gene Accession#: 15925401 and 15924364) strains by our approach. Gene GI#49484133 in MRSA 252 and GI#15924882 in Mu50 respectively represents *Staphylococcus aureus* murE gene encoding UDP-N-acetylmuramyl tripeptide synthetase, which was demonstrated to be essential in *Staphylococcus aureus* through a method incorporating an IPTG controllable promoter [42].

Although the cell wall has long been considered an attractive target for antibiotic development because of its absence in humans, what should not be overlooked is that one of the most common antibiotic resistance mechanisms is the metamorphosis of cell-wall proteins, resulting in inhibiting antibiotic activity. For example, β-lactam

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TABLE 2. 133 ESSENTIAL NON-HUMAN HOMOLOGOUS GENES IN BOTH MRSA 252 AND MU50 ENCODING DIFFERENT CLASSES OF PROTEINS AND THEIR PUTATIVE OR HYPOTHETIC FUNCTIONS

Categories	Classes	General Functions	MRSA 252 *NCBI Gene Accession#*	MRSAMu50 *NCBI Gene Accession #*	Specific putative or hypothetic functions
Metabolism	Cellular respiration	Carbohydrate Catabolism	49482458	15923216	Formate acetyltransferase
			49482459	15923217	Formate acetyltransferase activating enzyme
			49482486	15923242	Xylitol dehydrogenase
			49483017	15923750	HPr kinase/phosphorylase
			49483247	15924074	Phosphoenolpyruvate-protein phosphatase ptsI
			49483033	15923765	Phosphoglyceromutase
			49483952	15924701	Acetate kinase
			49484267	15925031	Sucrose-6-phosphate hydrolase
			49484349	15925115	Fructose-bisphosphate aldolase
			49484367	15925133	Mannose-6-phosphate isomerase
			49484381	15925149	Mannitol-1-phosphate 5-dehydrogenase
			49484415	15925185	Galactose-6-phosphate isomerase subunit LacA
		Lipid Catabolism	49483384	15924216	Phosphatase/ dihydroxyacetone kinase
			49483425	15924288	Glycerol uptake operon antiterminator regulatory protein
		Amino acid catabolism	49482426	15923174	N-acetyl-γ-glutamyl-phosphate reductase
			49482779	15923539	N-acyl-L-amino acid amidohydrolase
			49483163	15923990	Thimet oligopeptidase homolog
			49483313	15924141	Glutamate racemase
			49483846	15924589	5'-methylthioadenosine nucleosidase/S-adenosylhomocysteine nucleosidase
			49484504	15925279	Urease subunit β
			49484120	15924869	Aminopeptidase ampS
			49484649	15925422	Glycerate kinase
			49484868	15925663	HisF cyclase-like protein
				15923177	Cystein Hydrolase
			49483520	15924318	Homoserine dehydrogenase
			49483584	15924384	Aspartate semialdehyde dehydrogenase
				15925319	Amino acid amidohydrolase
		Common metabolic pathway	49482818	15923578	Phosphotransacetylase
			49484161	15924909	Putative manganese-dependent inorganic pyrophosphatase
			49484002	57634637	Probable NAD(FAD)-utilizing dehydrogenase
	Bio-synthesis	Amino acid biosynthesis	49484873	15925668	Histidinol dehydrogenase
			49482425	15923173	Ornithine acetyltransferase
			49482586	15923346	5-methyltetrahydropter-oyltriglutamatehomo- cysteine methyltransferase
			49482696	15923462	Glutamate synthase, large subunit
			49483565	15924362	Tryptophan synthase β subunit
			49483583	15924383	Aspartokinase II
			49483655	15924456	Chorismate synthase
			49484279	15925043	dihydroxy acid dehydratase
			49484281	15925046	Ketol-acid reductoisomerase
			4948429	15925060	Alanine racease
			49484794	15925588	Pantoate--β-alanine ligase
		Fatty acid biosynthesis	49483392	15924219	Fatty acid/phospholipid synthesis protein
		Nucleotide biosynthesis	49482382	15923129	Phosphopentomutase
			49483421	15924248	Uridylate kinase
			49483664	15924468	Cytidylate kinase
		Cell wall biosynthesis	49484627	15925401	FemAB family protein
			49483567	15924364	FemA protein
			49482490	15923244	Teichoic acid biosynthesis protein (truncated TagF)
			49482939	15923673	Undecaprenyl Pyrophosphate Phosphatase
			49482995	15923728	UDP-N acetylenolpyruvoyl-glucosamine reductase
			49483182	15924008	UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase
			49484307	15925072	UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate-D-alanyl-D-alanyl ligase
			49484133	15924882	UDP-N-acetylmuramyl tripeptide synthetase

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			49483346	15924173	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase
			49484348	15925114	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
			49484309	15925074	Rod shape determining protein RodA
			49483587	15924387	Tetrahydrodipicolinate acetyltransferase
			49483980	15924730	UDP-N-acetyl-muramoyl-L-alanine synthetase
				57634647	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
		Other biosynthesis	49482716	15923479	tetrapyrrole(corrin/porphy-rin) methylase
			49482722	15923485	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
			49484013	15924759	Riboflavin biosynthesis
			49484795	15925589	3-methyl-2-oxobutanoate hydroxymethyltransferase
Transmission of genetic information	DNA replication, recombination and repair		49482254	15922991	Chromosomal replication initiation protein
			49482255	15922992	DNA polymerase III β subunit
			49482269	15923006	Replicative DNA helicase (DnaB-like)
			49483309	15924136	Excinuclease ABC subunit C
			49483633	15924434	Methyltransferase
			49483747	15924487	Integrase/recombinase
			49483811	15924552	DNA primase
			49483834	15924577	DNA polymerase III subunit delta
			49483926	15924674	Primosomal protein DnaI
			49483944	15924693	DNA polymerase III, β chain
			49484385	15925153	DisA bacterial checkpoint controller nucleotide binding
	Transcription and RNA processing		49483418	15924245	Transcriptional repressor CodY
			49483550	15924347	Transcription antiterminator
			49484097	15924845	SpoU rRNA methylase family protein
			49484908	15925703	Ribonuclease P
			49483433	15924260	Ribosome-binding factor A
			49483855	15924600	Transcription elongation factor
			49482590	15923350	Transcription terminator
			49483976	15924726	Catabolite control protein A
	Translation and posttranslational modifications		49483000	15923733	peptidase T
			49483039	15923772	SsrA-binding protein
			49483384	15924211	Hypothetical translation and posttranslational modifications
			49483609	15924409	Gcn5-related acetyltransferases
			49483778	15924518	Elongation factor P
Trans-membrane Proteins	Antibiotic Resistance		49482275	15923012	Metallo- lactamase
			49483344	15924171	Penicillin-binding protein
	Regulation		49483168	15923996	GTP pyrophosphokinase
			49483425	15924252	Zinc metalloprotease yluc
	Transport		49482431	15923179	Glucose-specific PTS, IIABC component
			49482476	15923232	PTS, IIBC component
			49482956	15923690	Gructose-specific PTS, IIABC component
			49483966	15924716	N-acetylglucosamine specific PTS, IIABC component
			49484378	15925146	Mannitol-specific PTS, IIBC component
			49484380	15925148	Mannitol specific PTS, IIA component
			49484538	15925313	PTS, arbutin-like, IIBC component
			49484739	15925528	Glucose-specific PTS, II ABC component
			49484838	15925631	PTS, IIABC component
			49483148	15923977	Oligopeptide transport system permease protein
			49484706	15925495	Gluconate permease
			49482866	15923628	Teichoic acid ABC transporter permease
			49484434	15925210	Cobalt transport protein
			49484516	15925291	Na^+/H^+ antiporter
			49484891	15925688	Nickel transport protein
			49484846	15925639	Bifunctional Preprotein translocase subunit SecA
			49483881	15924627	Bifunctional preprotein translocase subunit SecD/SecF
			49483265	15924092	Spermidine/putrescine-binding protein precursor homolog
			49482314	15923062	Potassium-transporting ATPase subunit A
			49482353	15923100	L-lactate permease homolog
			49484303	15925067	potassium-transporting ATPase subunit A
			49484446	15925220	Preprotein translocase subunit SecY
			49483071	15923829	ABC transporter substrate-binding protein
			49483075	15923833	ABC transporter-associated protein
			49483078	15923836	ABC transporter-associated protein

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Other Proteins	Carrier proteins	49483175	15924003	Sodium/proton-dependent alanine carrier protein
		49482688	15923454	Lipoprotein
	Regulation	49482271	15923008	Response regulator protein
	Cell division	49482736	15923499	C ell division
		49483349	15924176	C ell division protein FtsZ
		49484905	15925700	Glucose-inhibited division protein B
	Other	49484374	15925142	Haloacid dehalogenase-like hydrolase
		49484612	15925386	Nitrate reductase β chain
		49484613	15925387	Respiratory nitrate reductase alpha chain
	Unknown function	49482472	15923228	Unknown
		49483005	15923738	Unknown
		49483022	15923755	Unknown
		49483024	15923757	Unknown
		49483035	15923767	Unknown
		49483546	15924343	Unknown
		49483928	15924676	Unknown
		49484792	15925584	Unknown

resistance was attributed to the expression of a group of cell wall penicillin-binding proteins (PBP-2') encoded by the mecA gene [43, 44]. Glycopeptide resistance is also considered to be caused by cell wall thickening resulting in binding vancomysin extracellularly [45,46] and/or alteration of the drug-acting site in the cell wall from D-alanine-D-alanine to D-alanine-D-lactate owing to the expression of *vanA* resistance gene [47]. Hence, for novel antibiotic development, substances that anchor in sites other than the bacterial cell wall may have more potential because resistance usually arises as the result of gene mutation on the target proteins that are subject to direct antibiotic attack [48]. A 2006 review on mechanisms of bacterial antibiotic resistance suggested the exploration of novel antibiotics with alternative mechanisms of action [49].

Genes involved in transmission of genetic information including DNA replication, recombination and repair, transcription and RNA processing, translation, post-translational modification remain viable targets for antibacterial agent development [33]. Our approach identified 24 of these candidate genes.

Our approach identified 29 membrane bound proteins. A recent review on anti-MRSA drug development indicated that agents anchoring in the bacterial membrane (*e.g.,* ceragenins and lipopeptides) showed great bactericidal effect and may be less prone to drug resistance due to the incapability of bacteria to modify their targets in a way that is compatible with their survival [50]. Among this pool of proteins, 19 are involved in membrane transport, which represent valid drug targets because pathogens usually lose their biosynthetic capabilities and rely on their hosts for the supply of essential nutrients [51, 52].

Our approach identified 30 energy metabolic (*i.e.* cellular respiration) genes in both MRSA 252 and MRSA Mu50, which are essential to staphylococcal survival with *E*-score < 10^{-4} but absent in human genome with *E*-score <10^{-3}. Currently there are limited numbers of commercially available antibiotics targeting energy metabolism. Those existing are mainly biological reagents such as oligomycin [53] and pesticides or piscicides such as antimycin A [54], not commonly used for humans in that they affect both bacterial and human cells. Surprisingly, nature has provided us with a group of energy metabolic enzymes which are essential to pathogen survival while absent in humans. The differentiation lies in that those enzymes function through alternative mechanisms other than their counterpart enzymes in humans. For example, fructose-1, 6-diphosphate aldolase (FBPA) is one of the key enzymes in the glycolytic pathway that involves the breakdown of glucose [55]. FBPA is divided into two classes based on structural properties and catalytic mechanisms [56]. Class I FBPA is mainly found in higher order organisms (*e.g.,* human and animals). Catalysis in Class I FBPA proceeds via a Schiff base intermediate formed by an active site lysine residue [55]. Class II FBPA is usually found in yeasts, bacteria, fungi, and parasites [56]. Catalysis in Class II FBPA centers on the participation of a Zn (II) cofactor that coordinates to an enolate anion intermediate [54]. Multiple alignment of the sequence of *MRSA* FBPA with class II *giardia* FBPA and class I *human* FBPA was shown in Fig. 1. *MRSA* FBPA (NCBI Gene Accession[#]: 49484349 and 15925115 respectively) exhibits 40.8% sequence identity to Class II *giardia* FBPA while it exhibits only 18.8 % sequence identity to class I *human* FBPA [57,58]. Thus, *MRSA* FBPA should be hypothetically classified into class II FBPA, not class I FBPA. Validation of the essential nature of class II *MRSA* FBPA through allelic replacement and inducible expression is underway in our research group. Based on major differences in active site structure and catalytic mechanism, an inhibitor of class II FBPA can be designed which will not inhibit class I FBPA.

Accumulating *in vitro* [59] and *in vivo* [60] evidence suggests that enzymes catalyzing bacterial cellular respiration with differentiated mechanisms of action are promising targets for novel antibiotic development. The

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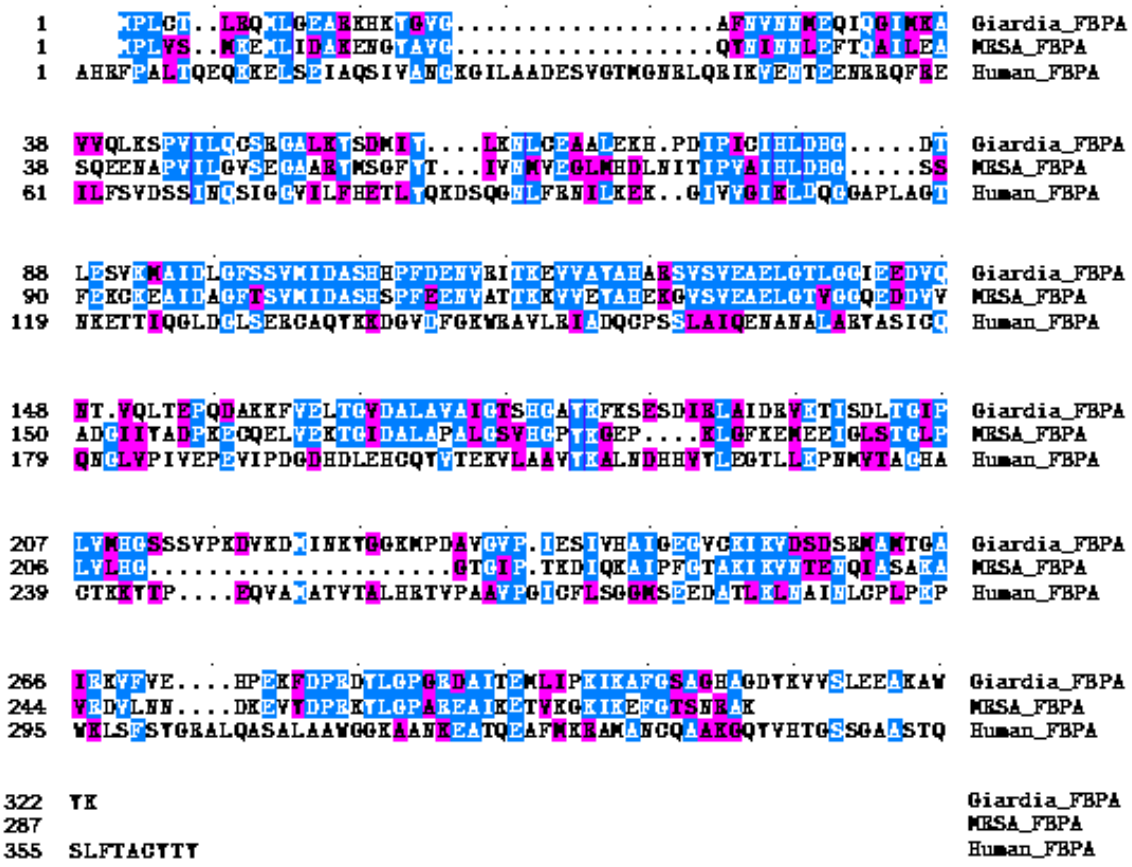

Figure.1 Alignment of the amino acid sequences of *MRSA* FBPA (NCBI GENE ACCESSION#:49484349 and 15925115 respectively) with class II *giardia* FBPA (2ISV) and class I *human* FBPA(1QO5). Numbering of the amino acids is indicated on the left. Identical amino acid residues in the alignment are indicated in light-blue shading and similar amino acid residues are indicated in purple shading. Gaps introduced during the alignment process are indicated as dotes.

inhibitors designed are able to hinder bacterial growth by inhibition of those enzymes without interfering with their human cousins. Most importantly, attacking bacterial energy-making machinery bypasses the usual bacterial mutation sites for drug resistance [61-62]. The rationale lies in that almost all existing antibiotics target only 4 cellular functions: cell wall synthesis, protein synthesis, nucleic acid synthesis and foliate synthesis, though there are hundreds of antibiotics on the market [63]. Repeated exposure of bacteria to antibacterial reagents targeting similar sites increases the chance of bacterial gene mutation, which remains to be the primary cause of the prevalence of antibiotic-resistant bacteria, such as MRSA, NDM-1 induced antibiotic -resistant *Escherichia coli* [62], and *etc*.. Exploration of antibiotics targeting alternative cellular functions such as central metabolic pathways may be a promising direction, and selective inhibition of targets specific to bacterial energy metabolism may be a potentially efficacious alternative in the treatment of MRSA infections. The enzymes on the higher priority list include *MRSA* FBPA, *MRSA* dihydroxyacetone kinase (DAK) 2 Phosphatase, *MRSA* acetate kinase, *MRSA* histidinol dehydrogenase, *MRSA* Phosphotransacetylase, *MRSA* Sucrose-6-phosphate hydrolase and *MRSA* glycerate kinase, which either do not have human homologues or adopt dramatically different catalytic mechanisms comparing to their human cousins.

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CONCLUSION AND FUTURE WORK

One of the crucial steps in narrow-spectrum antibiotics development is target identification. In this study, a putative set of candidate drug targets were elucidated by an in silico approach. The candidate genes are hypothetically required for survival of the candidate microorganism and have no close human analogue. Many identified targets have been experimentally validated [41-44, 65-68]. By shortening the list of potential drug targets to a small pool of genes, the data presented in this paper facilitated our group and, may also aid other researchers in pursuing target validation and target characterization for alternative treatment of MRSA infections. Future directions include developing inhibitors. for the candidate proteins. In principle, the premise is that the inhibitors of these targets should only be toxic to pathogens but safe for use by humans.

More importantly, we propose that a class of central metabolic enzymes, such as MRSA FBPA, MRSA dihydroxyacetone kinase (DAK) 2 Phosphatase, MRSA acetate kinase, MRSA histidinol dehydrogenase, MRSA Phosphotransacetylase, MRSA Sucrose-6-phosphate hydrolase and MRSA glycerate kinase (table 2), are promising antibiotic drug targets due to the fact that they either do not have their human counterparts or if they do, different catalytic mechanisms are employed (*e.g.,* class I and class II FBPA). Based on major differences in active site structure and catalytic mechanism, an inhibitor of such a bacterial enzyme can be designed which will not inhibit its human cousin. Also, the risk of bacterial drug resistance against inhibitors of those enzymes may be low because antibiotics targeting bacterial central metabolism are not commonly used. Those cellular sites are not repeatedly exposed to antibacterial agents thus less prone to drug resistance. Proposed long-term work includes utilizing MRSA as a model bacterial system to develop methods combating antibiotic resistance. It is even more crucial that this type of investigation is undertaken in academia than it would be if industry were still heavily investing in it [24, 63].

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REFERENCES

[1] M. E. Mulligan, K. A. Murray-Leisure, B. S. Ribner, H. C. Standiford, J. F. John, J. A. Korvick, C. A. Kauffman and V. L. Yu, "Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management," Am. J. Med. vol.94, Mar. 1993, pp. 313-328.

[2] J. M. Voyich, M. Otto, B. Mathema, K. R. Braughton, A. R. Whitney, D. Welty, R. D. Long, D. W. Dorward, D. J. Gardner, G. Lina, B. N. Kreiswirth and F. R. DeLeo, "Is panton-valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease?" J. Infect. Dis. vol.194, Dec. 2006, pp. 1761-1770.

[3] Centers for Disease Control and Prevention, (2007). MRSA: Methicillin-resistant *Staphylococcus aureus* in Healthcare Settings.

[4] R. M. Klevens, M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey and S. K. Fridkin, "Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States," JAMA, vol. 298, Oct. 2007, pp. 1763-1771.

[5] M. Jevons, Celbenin"-resistant staphylococci, Br. Med. J., vol. 1, 1961, pp.124-125.

[6] T. Foster, (1996). *Staphylococcus*. In: Barron's Medical Microbiology (Barron S *et al*, eds.), 4th ed. Galveston, TX.

[7] K. Okuma, K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito and K. Hiramatsu, "Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community", J. Clin. Microbiol. vol. 40, Nov. 2002, pp. 4289–4294, doi: 10.1128/JCM.40.11.4289-4294.2002

[8] H. Huang, N. M. Flynn, J. H. King, C. Monchaud, M. Morita and S. H. Cohen, "Comparisons of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Hospital-Associated MSRA Infections in Sacramento, California," J. Clin. Microbiol. vol. 44, July 2006, pp. 2423-2427, doi:10.1128/JCM.00254-06

[9] R. C. Moellering, "Vancomycin: A 50-Year Reassessment," Clin. Infect. Dis. vol. 42, Suppl 1, Jan. 2006, pp. S3-4.

[10] P. L. Donald, "Vancomycin: A History," Clin. Infect. Dis. vol., 42, Suppl 1, Jan. 2006, pp. S5-S12.

[11] G. C. Schito, "The importance of the development of antibiotic resistance in *Staphylococcus aureus*," Clin. Microbiol. Infect., Suppl 1, Mar. 2006, pp. 16445718.

[12] K. Sieradzki and A. Tomasz, "Inhibition of cell wall turnover and autolysis by vancomycin in a highly vancomycin-resistant mutant of *Staphylococcus aureus*," J. Bacteriol., vol. 179, April, 1997, pp. 2557-2566.

[13] C. Burlak, C. H. Hammer, M. Robinson, A. R. Whitney, M. J. McGavin, B. N. Kreiswirth and F. R. DeLeo, "Global analysis of community-associated methicillin-resistant *Staphylococcus aureus* exoproteins reveals molecules produced *in vitro* and during infection Cell" Microbiol., vol. 9, Jan. 2007, pp.1172–1190, doi:10.1111/j.1462-5822.2006.00858.x

[14] J. D. Siegel, E. Rhinehart, M. Jackson and L. Chiarello, (2008). Management of multi-drug resistant organisms in healthcare settings, 2006. US Centers for Disease Control and Prevention. Healthcare Infection Control Practices Advisory Committee. Accessed January 25.

[15] L. Nicolle, "Community-acquired MRSA: a practitioner's guide," CMAJ., vol. 175, June 2006, pp. 145, doi:10.1503/cmaj.060457

 ISBN: 978-1-61208-137-3

[16] Centers for Disease Control and Prevention. Epidemiology and management of MRSA in the Community. October 26, 2007. Accessed January 25, 2008.
[17] J. A. Gorchynski and J. K. Rose, "Complications of MRSA Treatment: Linezolid-induced Myelosuppression Presenting with Pancytopenia," Western Journal of Emergency Medicine, vol. 9, August 2008, pp. 177-178
[18] Cubist Pharmaceuticals, Inc. (2003). Daptomycin (Cubicin) package literature. Cubist Pharmaceuticals, Inc., Lexington, Mass.
[19] R. Moellering, Trends in New Drug Development; from Broad- to Narrow-Spectrum Antibiotics Program and abstracts from the 39th ICAAC Symposium 138, F 1363 .
[20] F. Tally, Trends in New Drug Development; from Broad- to Narrow-Spectrum Antibiotics Program and abstracts from the 39th ICAAC Symposium 138, F 1364 .
[21] Y. Zhang, "The Magic Bullets and Tuberculosis Drug Targets. Ann Rev Pharmacol Toxicol, " vol. 45, 2005, pp. 529-564, doi: 10.1146/annurev.pharmtox.45.120403.100120
[22] J. Smith and V. Stein, "SPORCalc: A development of a database analysis that provides putative metabolic enzyme 2008. reactions for ligand-based drug design," Comput. Biol. Chem., vol. 33, Apr.2009, pp.149–159, doi:10.1016/j.compbiolchem.2008.11.002
[23] W. F. de Azevedo and R. Dias, "Computational methods for calculation of ligand-binding affinity". Curr Drug Targets vol.9, Dec. 2008, pp. 1031–1039.
[24] S. Projan, "Why is big Pharma getting out of antibacterial drug discovery?" Curr. Opin. Microbiol. vol. 6, Oct. 2003, pp. 427–430, doi:10.1016/j.mib.2003.08.003
[25] P. F. Boreham, R. E. Phillips and R. W. Shepherd, "Altered uptake of metronidazole in vitro by stocks of Giardia intestinalis with different drug sensitivities," Trans. R. Soc. Trop. Med. Hyg. vol. 82, May 1988, pp. 104-106.
[26] S. D. Mills, "The role of genomics in antimicrobial discovery," J. Antimicrob. Chemother., vol. 51, Mar. 2003, pp.749-752, doi: 10.1093/jac/dkg178
[27] K. R. Sakharkar, M. K. Sakharkar and V. T. Chow, "A novel genomics approach for the identification of drug targets in pathogens, with special reference to *Pseudomonas aeruginosa*," *In Silico* Biology, vol. 4, 2004, pp.355-360.
[28] V. Sharma, P. Gupta, and A. Dixit, "Identification of putative drug targets from different metabolic pathways of *Aeromonas hydrophila*," *In Silico.* Biology," vol 4, 2008, pp. 331-338.
[29] http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi(03/15/2011)
[30] W. Li, L. Jaroszewski and A. Godzik, "Clustering of highly homologous sequences to reduce the size of large protein databases," Bioinformatics, vol. 17, Mar. 2001, pp. 282-283, doi: 10.1093/bioinformatics/17.3.282
[31] R. Zhang, H. Ou and C. Zhang, "DEG, a Database of Essential Genes," Nucleic Acids Res. vol. 32, (suppl 1), Jan. 2004, pp. D271-D272, doi: 10.1093/nar/gkh024
[32] R. Zhang and Y. Lin, "DEG 5.0, a database of essential genes in both prokaryotes and eukaryotes," Nucleic Acids Res., vol. 37, (suppl 1): Jan. 2009, pp. D455-D458, doi: 10.1093/nar/gkn858
[33]http://www.ebi.ac.uk/integr8/InquisitorPage.do (03/15/2011)
[34] B. A. Diep, H. A. Carleton, R. F. Chang, G. F. Sensabaugh and F. Perdreau-Remington, "Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*," J. Infect. Dis. vol. 193, Apr.2006, pp.1495–1503, doi: 10.1086/503777
[35] A. P. Johnsona, H.M. Auckenb, S. Cavendishc, M. Gannerb, M. C. J. Walec, M. Warnera, D. M. Livermorea and B. D. Cooksonb "Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System" (EARSS)". J. Antimicrob. Chemother., vol. 4, 2001, pp. 143–144, doi:10.1093/jac/48.1.143.
[36] K. Hiramatsu, N. Aritaka and H. Hanaki, "Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin," Lancet, vol. 350, Dec. 1997, pp. 1670-1673, doi:10.1016/S0140-6736(97)07324-8
[37] E. S. Lander, L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, *et al.*, "Initial sequencing and analysis of the human genome," Nature vol. 409, Feb. 2001, pp. 860-921, doi:10.1038/35057062
[38] J. C. Venter, M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, *et al.*, "The sequence of the human genome" Science, vol. 291, Feb. 2001, pp.1304-1351,doi: 10.1126/science.1058040.
[39] K. L. Longenecker, G. F. Stamper, P. J. Hajduk, E. H. Fry, C. G. Jakob, J. E. Harlan, R. Edalji, D. M. Bartley, K. A. Walter, L. R. Solomon, T. F. Holzman, Y. G. Gu, C. G. Lerner, B. A. Beutel and V. S. Stoll, "Structure of MurF from *Streptococcus pneumoniae* co-crystallized with a small molecule inhibitor exhibits interdomain closure," Protein Sci., vol. 14, Dec. 2005, pp3039-3047, doi: 10.1110/ps.051604805
[40] A. Perdih, M. Kotnik, M. Hodoscek and T. Solmajer, "Targeted molecular dynamics simulation studies of binding and conformational changes in *E. coli* MurD," Proteins, vol.68, Apr. 2007,pp. 243-254. doi: 10.1002/prot.21374
[41] A. Strandén, K. Ehlert, H. Labischinski and B. Berger-Bächi, "Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in a femAB null mutant of methicillin-resistant *Staphylococcus aureus*," J. Bacteriol. vol. 179, Jan. 1997, pp. 9–16.
[42] J. Malabendu, T. Luong, H. Komatsuzawa, M. Shigeta and C. Y. Lee, "A method for demonstrating gene essentiality in *Staphylococcus aureus*," Plasmid, vol. 44, Mar. 2000. pp. 100-104, doi:10.1006/plas.2000.1473
[43] N. H. Georgopapadakou and F. Y. Liu, "Binding of β-lactam antibiotics to penicillin-binding proteins of *Staphylococcus aureus* and *Streptococcus faecalis*: relation to antibacterial activity," Antimicrob. Agents Chemother. vol. 18, Nov. 1980, pp.834-836.
[44] K. Ubukata, N. Yamashita and M. d Konno, "Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin resistant *staphylococci*," Antimicrob. Agents Chemother. vol. 27, May 1985, pp. 851-857.
[45] M. Kuroda, H. Kuroda, T. Oshima, F. Takeuchi, H. Mori and K. Hiramatsu, "Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*," Mol. Microbiol. vol.49, Aug. 2003, pp807–821, doi:10.1046/j.1365-2958.2003.03599.x
[46] L. Cui, H. Murakami, K. Kuwahara-Arai, H. Hanaki and K. Hiramatsu, "Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50," Antimicrob. Agents Chemother. vol. 44, Sep. 2000, pp. 2276–2285.
[47] A. Severin, K. Tabei, F. Tenover, M. Chung, N. Clarke and A. Tomasz, "High level oxacillin and vancomycin resistance and altered cell wall composition in *Staphylococcus aureus* carrying the staphylococcal mecA and the enterococcal vanA gene complex," J. Biol. Chem. vol. 279, Jan. 2004, pp. 3398-3407.
[48] A. A. Salyers, and D. D. Whitt, (2005). Revenge Of The Microbes: How Bacterial Resistance Is Undermining The Antibiotic Miracle, American Society for Microbiology Press, Washington, DC.
[49] F. C. Tenover, "Mechanisms of antimicrobial resistance in bacteria," Am J Med., vol. 119 (6 Suppl 1), June 2006, pp. S3–S10, doi:10.1016/j.ajic.2006.05.219

 ISBN: 978-1-61208-137-3

[50] F. Van Bambeke, M. P. Mingeot-Leclercq, M. J. Struelens and P. M. Tulkens, "The bacterial envelope as a target for novel anti-MRSA antibiotics," Trends Pharmacol Sci. vol. 29, Mar. 2008, pp. 124-134.

[51] K. Lewis, "Multidrug resistance: versatile drug sensors of bacterial cells," Curr. Biol., vol. 9, Jun. 1999, pp. R403-R407, doi:10.1016/S0960-9822(99)80254-1

[52] C. M. Fraser, J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, R. D. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Saudek, C. A. Phillips, J. M. Merrick, J. F. Tomb, B. A. Dougherty, K. F. Bott, P. C. Hu, T. S. Lucier, S. N. Peterson, H. O. Smith, C. A. Hutchison 3rd and J. C. Venter, "The minimal gene complement of *Mycoplasma genitalium*," Science. vol. 270, Oct. 1995, pp. 397-403, DOI: 10.1126/science.270.5235.397

[53] Y. Kagawa and E. Racker, "Partial resolution of the enzymes catalyzing oxidative phosphorylation. 8. Properties of a factor conferring oligomycin sensitivity on mitochondrial adenosine triphosphatase," J. Biol. Chem., vol.241, May 1966, pp. 2461-2466.

[54] D. Xia, C. H. Yu, H. Kim, J. Xia, A. M. Kachurin, L. Zhang, L. Yu and J. Deisenhofer, "Structure of Antimycin A1, a Specific Electron Transfer Inhibitor of Ubiquinol-Cytochrome c Oxidoreductase" J. Am. Chem. Soc., vol. 121, Aug. 1999, pp.4902–4903, DOI: 10.1002/chin.199933269

[55] T. Gefflaut, C. Blonski, J. Perie and M. Willson, "Class I aldolases: substrate specificity, mechanism, inhibitors and structural aspects," Prog. Biophys. Mol. Biol. vol.63, 1995, pp. 301-340.

[56] A. Galkin, L. Kulakova, E. Melamud, L. Li, C. Wu, P. Mariano, D. Dunaway-Mariano, T. E. Nash and O. Herzberg, "Characterization, Kinetics, and Crystal Structures of Fructose-1,6-bisphosphate Aldolase from the Human Parasite *Giardia lamblia,"* J. Biol. Chem., vol. 282, Feb. 2007, pp. 4859–4867.

[57] S. F. Altschul, T. L. Madden, A. A. Schäffe, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. vol. 25, Sep. 1997, pp. 3389-3402.

[58] J.D. Thompson, D.G. Higgins and T.J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice," Nucleic Acids Res. vol. 22, Nov. 1994, pp. 4673-4680, doi: 10.1093/nar/22.22.4673.

[59] A. Fredenhagen, S. Y. Tamura, P. T. M. Kenny, H. Komura, Y. Naya, K. Nakanishi, K. Nishiyama, M. Sugiura and H. Kita, "Andrimid, a new peptide antibiotic produced by an intracellular bacterial symbiont isolated from a brown planthopper" J. Am. Chem. Soc. vol. 109, Jul. 1987, pp. 4409–4411, doi: 10.1021/ja00248a055

[60] C. Freiberg, J. Pohlmann, P. G. Nell, R. Endermann, J. Schuhmacher, B. Newton, M. Otteneder, T. Lampe, D. Häbich and K. Ziegelbauer, "Novel bacterial acetyl coenzyme A carboxylase inhibitors with antibiotic efficacy *in vivo,"* Antimicrob Agents Chemother, vol. 50, Aug. 2006, pp. 2707-2712.

[61] F. R. Stermitz, P. Lorenz, J. N. Tawara, L. A. Zenewicz and K. Lewis, "Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor," *Proc. Natl. Acad. Sci. U.S. A.* vol. 97, Feb. 2000, pp. 1433-1437.

[62] N. R. Guz and F. R. Stermitz, "Synthesis and structures of regioisomeric hydnocarpin-type flavonolignans," *J. Nat. Prod.* Vol. 63, Aug. 2000, pp. 1140-1145, DOI: 10.1021/np000166d.

[63] C. Nathan, "Antibiotics at the crossroads," Nature, vol. 431, Oct. 2004, pp. 899–902, doi :10.1038/431899a

[64] K. K. Kumarasamy, M. A. Toleman, T. R. Walsh, J. Bagaria, F. Butt, R. Balakrishnan, and U. Chaudhary, *et al.* "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study". Lancet Infect Dis vol.10, August 2010, pp. 597–602, doi:10.1016/S1473-3099(10)70143-2.

[65] A. Boniface, A. Bouhss, D. Mengin-Lecreulx and D. Blanot, "The MurE synthetase from Thermotoga maritima is endowed with an unusual D-lysine adding activity," J. Biol. Chem. vol. 281, Jun. 2006, pp. 15680-15686.

[66] T. Deva, E. N. Baker, C. J. Squire and C. A. Smith, "Structure of *Escherichia coli* UDP-N-acetylmuramoyl:L-alanine ligase (MurC)," Acta. Crystallogr. D. Biol. Crystallogr. vol.62, Dec. 2006, pp. 1466-1474, doi:10.1107/S0907444906038376

[67] C. A. Smith, "Structure, function and dynamics in the mur family of bacterial cell wall ligases," J. Mol. Biol. vol. 362, Sep.2006, pp. 640-655, doi:10.1016/j.jmb.2006.07.066.

[68] K. Ehlert, "Methicillin-resistance in *Staphylococcus aureus* - molecular basis, novel targets and antibiotic therapy," Curr. Pharm. Des. vol.5, Feb.1999, pp. 45-55.

 ISBN: 978-1-61208-137-3