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RESEARCH ARTICLE

BIOINFORMATICS

EXPLORING BIOLOGICAL NETWORKS FOR FINDING POTENTIAL DRUG TARGETS IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a dreadful pathogen causing broad range of infections. The complexity in treating these pathogens corresponds to the alarming increase and spread of resistance against antibiotics. Thus the search for novel antimicrobials targeting towards new drug targets becomes indispensable for combating such resistant pathogens. The present study focuses on exploring the essential bacterial pathways of MRSA for the identification of promisable drug targets with the aid of metabolic pathway analysis and choke point analysis. The present work thus paves the way for treating MRSA with new antimicrobials targeted towards the identified promisable drug targets.

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KEYWORDS

Methicillin resistant *Staphylococcus aureus,* Antimicrobials, Metabolic pathway analysis and Choke point analysis

INTRODUCTION

The discovery of truly miraculous drugs, the antibiotics, is a landmark medical advance in the history of medicine. The continuous new armamentarium of antibiotics leads to the successful management of most of the infectious diseases from the beginning of 20th century [1]. Despite the advances in antimicrobial and vaccine development, the era of antibiotics is threatened by the emergence of resistant pathogens [2].

Staphylococcus aureus, a member of the family Micrococcaceae, is a gram positive opportunistic pathogen responsible for a series of life threatening infections such as septicemia, endocarditis and toxic shock syndrome (TSS) [3]. Their pathogenecity corresponds to their property of acquiring new exogenous genes; thereby increasing their propensity to acquire resistance to multiple antimicrobial agents [4].

Recent studies revealed that the mortality due to Methicillin resistant *Staphylococcus aureus* (MRSA) in the United States exceeds that from human immuno deficiency virus infections and AIDS [5]. The increased prevalence of MRSA and its growing phenomenon of bacterial resistance caused by the overuse and misuse of antibiotics leads to simultaneous decline in the research and development of novel products [6].

It is indisputable that new drugs, notably antibiotics are in critical demand to halt and reverse the relentless spread of antibiotic resistance pathogens like MRSA which cause life threatening diseases [7]. The use of computational methods in the early stages of target identification and drug discovery becomes more attractive to solve the pressing need of combating the pathogens, which becomes resistant to antimicrobial agents [8].

Metabolic pathway analysis of large and highly entangled networks is used to investigate novel drug targets in pathogenic organisms [9]. Choke point analysis can be best utilized for the discovery of potential drug targets in the metabolic network of various pathogens with the advantage of testing the consistency between the experimental data and the assumptions on regulation of biochemical pathways [10]. Choke point enzymes are crucial in the metabolic pathway of any organism and are those that take part in a reaction that consumes a specific substrate or uniquely produces a specific product. Inactivation of these enzymes leads to the disruption of the metabolic network of the corresponding organism [11].

Thus the present study focuses on getting insights into the different metabolic pathways for the identification of potential drug targets in methicillin resistant *Staphylococcus aureus* by choke point analysis.

MATERIALS AND METHODS

i) Retrieval of metabolic pathways:

The metabolic pathway analysis was done by KEGG Automatic Annotation Server (KAAS) (Ogata *et al*., 1999). The complete set of metabolic pathways of *Homo sapiens* and Methicillin resistant *Staphylococcus aureus* were obtained from pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG).

ii) Comparison of pathways:

The pathways of *Homo sapiens* and Methicillin resistant *Staphylococcus aureus* were compared and those that are present in Methicillin resistant *Staphylococcus aureus* but not in *Homo sapiens* were considered to be the pathogen specific pathways.

iii) Search for promisable enzymes:

The pathways that are exclusive for Methicillin resistant *Staphylococcus aureus* was searched for promisable enzymes. The promisable enzymes have been selected based on the choke point analysis. Choke point enzymes are crucial in the metabolic pathway of any organism and are those that take part in a reaction that consumes a specific substrate or uniquely produces a specific product. Inactivation of these enzymes leads to the disruption of the metabolic network of the corresponding organism [11].Choke point analysis can be performed using Pathway Hunter Tool [12].

iv) Similarity search:

The promisable enzymes were searched for the presence of any detectable homologous proteins in *Homo sapiens* using Blastp. The Blastp search against non-redundant database with the e-value inclusion threshold set at 0.005 was performed with the search restricted to proteins from *Homo sapiens* [13].

v) Analysis of targets for its essentiality:

The promisable targets thus obtained were subjected to DEG database search to confirm its essentiality to pathogen. Essential genes are those that constitute minimal gene set and are necessary for the survival of the organism [14].

vi) Exploring the biological significance of targets:

Predicting the localization of the proteins plays a crucial role in determining its function and facilitating their purification [15]. Thus the distribution of the essential targets pertaining to its significance was analyzed using PSORTb Subcellular Localization Prediction Tool [16].

RESULTS AND DISCUSSION

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database is found to contain 95 pathways pertaining to Methicillin resistant *Staphylococcus aureus* and 237 pathways pertaining to *Homo sapiens*. On comparing those pathways, it is revealed that *Homo sapiens* are devoid of 23 pathways that are found in Methicillin resistant *Staphylococcus aureus*. Hence these pathways are considered unique to Methicillin resistant *Staphylococcus aureus.* Out of these, 23 pathways, pathways like peptidoglycan biosynthesis and two-component system are considered to be important as they are inadequate for the survival of the Methicillin resistant *Staphylococcus aureus*. The other pathways such as geraniol degradation, caroteniod biosynthesis etc., are found to be not much essential for the existence of the organism and few metabolites of those pathways can be obtained through alternative pathways or sources. Hence only those unique pathways are considered for further analysis.

Table.1
Unique pathways of Methicillin resistant Staphylococcus aureus

S.No.	Pathway ID	Pathway
1	sar00281	Geraniol degradation
2	sar00312	beta-Lactam resistance
3	sar00362	Benzoate degradation
4	sar00401	Novobiocin biosynthesis
5	sar00473	D-Alanine metabolism
6	sar00521	Streptomycin biosynthesis
7	sar00550	Peptidoglycan biosynthesis
8	sar00621	Dioxin degradation
9	sar00624	Polycyclic aromatic hydrocarbon degradation
10	sar00625	Chloroalkane and chloroalkene degradation
11	sar00626	Naphthalene degradation
12	sar00627	Aminobenzoate degradation
13	sar00642	Ethylbenzene degradation
14	sar00660	C5-Branched dibasic acid metabolism
15	sar00680	Methane metabolism
16	sar00903	Limonene and pinene degradation
17	sar00906	Carotenoid biosynthesis
18	sar01110	Biosynthesis of secondary metabolites
19	sar01120	Microbial metabolism in diverse environments
20	sar02020	Two-component system
21	sar02060	Phosphotransferase system (PTS)
22	sar03070	Bacterial secretion system
23	sar05150	*Staphylococcus aureus* infection

Choke point analysis was performed for all the enzymes of peptidoglycan biosynthesis. Out of the 10 enzymes that possess a definite reaction step, 5 enzymes are found to be choke point enzymes in Methicillin resistant *Staphylococcus aureus* whose inactivation leads to the pathogens' failure to produce or consume a particular metabolite which could cause serious threat for fitness or survival of the same. The list of 10 enzymes involved in peptidoglycan biosynthesis with their enzyme ID, gene ID, reaction ID, load value (in), load value (out), presence or absence of choke point, K-shortest path (in) and K-shortest path (out) determined using Pathway Hunter tool is tabulated in Table.2

Table.2

List of 10 enzymes involved in peptidoglycan biosynthesis pathway of Methicillin resistant Staphylococcus aureus ranked by number of shortest paths.

Enzyme ID	Enzyme name	Gene ID	Reaction ID	LV (IN)	LV (OUT)	CP	k-SP (IN)	k-SP(OUT)
2.7.8.13	phospho-N-acetylmuramoyl-pentapeptide-transferase	SAR1158	R05629	1.79	2.02	+	71759	71759
6.3.2.10	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-alanyl-D-alanyl ligase	SAR2169	R04573	2.38	3.38	+	70165	70165
2.4.1.227	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	SAR1430	R05032	1.11	1.17	+	43501	43501
6.3.2.7	UDP-N-acetylmuramoylalanyl-D-glutamate--L-lysine ligase	SAR0988	R02786	1.3	2.31	–	31996	31996
2.5.1.7	UDP-N-acetylglucosamine 1-carboxyvinyl transferase	SAR2188	R00660	-0.83	-0.83	+	28392	28392
6.3.2.9	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	SAR1159	R02783	1.83	1.83	–	28058	28058
6.3.2.4	D-alanyl-alanine synthetase A	SAR2170	R01150	2.69	2	+	23526	23526
6.3.2.8	UDP-N-acetylmuramate--L-alanine ligase	SAR1818	R03193	0.88	0.88	–	10872	10872
1.1.1.158	UDP-N-acetylpyruvoylglucosmaine reductase	SAR0792	R03192	1.84	1.84	+	7698	7698
3.6.1.27	undecaprenyl pyrophosphate phosphatase	SAR0736	R05627	-1.5	-1.87	–	7037	7037

LV-Load Value; SP- Shortest Path; CP- Choke Point

Among the 5 choke point enzymes, UDP-N-Acetyl glucosamine 1- carboxy vinyl transferase (murA1) is considered as a promisable one which catalyzes the first committed step in peptidoglycan biosynthesis.

Besides these pathway based analysis, intensive literature search revealed the role of

signal transduction histidine-protein kinase involved in two-component system, chorismate synthase involved in shikimate pathway and 5'methylthioadenosine/S-adenosyl homocysteine nucleosidase involved in bacterial quorum sensing, as a promisable drug target.

Hence the choke point analysis is performed for all those enzymes and the results are tabulated in Table. 3.

Table.3
Choke point analysis of explored targets

S.NO.	Enzyme ID	Enzyme name	Gene ID	Reaction ID	LV (IN)	LV (OUT)	K-SP (IN)	K-SP (OUT)
1	4.2.3.5	Chorismate synthase	SAR1477	R01714	0.494	2.351	41589	41589
2	2.7.13.3	Sensor histidine kinase	SA1426	–	–	–	–	–
3	3.2.2.9	S-adenosylhomocysteine nucleosidase	SAR1676	R00194	-0.17	1.199	42048	42048
4	2.5.1.7	UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	SAR2188	R00660	-0.01	-0.83	28392	28392

On performing BlastP search against non-redundant database restricted to *Homo sapiens* subset with the e-value inclusion threshold set to 0.005, these enzymes are identified as non-homologous to human protein sequence. Further analysis with DEG server proposes these enzymes as potential targets and could be considered for drug design.

The Sub- cellular localization of the target protein is determined through PSORTb Subcellular Localization Prediction Tool. Three of the target proteins, UDP-N-acetylglucosamine 1-carboxyvinyl transferase 1, 5'-methylthioadenosine and chorismate synthase are found to be located in cytoplasm, while the two-component sensor histidine kinase protein is found to be located in cytoplasmic membrane. As the proteins are found to be located in cytoplasmic or cytoplasmic membrane, they are druggable and hence are considered as better drug targets.

Chorismate synthase is involved in the shikimate pathway catalyzing 1,4-trans elimination of phosphate from EPSP to yield chorismate, a precursor for synthesizing aromatic aminoacids, naphtoquinones, menaquinones and mycobactins. The shikimate pathway links the metabolism of carbohydrates to the biosynthesis of aromatic aminoacids. This pathway is not found in humans but seems to be essential for algae, fungi, bacteria higher plants and parasites and hence the shikimate pathway

enzymes can act as potential targets for antimicrobials [17, 18].

Two-component signal transduction system facilitates bacteria to sense, response and adapt to the changes in environment, stress and growth conditions. Sensor histidine kinase catalyzes its autophosphorylation and then subsequently transfers the phosphoryl group to a response regulator which leads to the changes in cellular physiology by regulating the gene expression [19]. As two-component system forms the integral elements of the virulence responses of pathogenic bacteria, they may act as the best target for the development of novel antimicrobial agents [20].

5'methylthioadenosine/S-adenosyl homocysteine hydrolase (MTAN) catalyzes the hydrolytic deadenylation of its substrate to form adenine and 5-metylthioribose or S-ribosylhomocysteine. Inhibition of MTAN affects adenine and methionine salvage, polyamine biosynthesis and quorum sensing thereby disrupting the growth and pathogenecity of bacteria. It is also found that MTAN is absent in mammals thereby making it as a better target for antibiotic design [21].

The bacterial cell wall is a unique structure in prokaryotes and is found to be an attractive target for novel antibiotics. The first committed step in peptidoglycan biosynthesis is the transfer of an enolpyruvate residue from phopsphoenolpyruvate to position 3 of UDP-N-acetylglucosmaine catalyzed by the enzyme, UDP-N-acetylglucosamine 1-carboxyvinyltransferase (murA). The absence of peptidoglycan biosynthesis pathway in mammals and disruption of bacterial cell integrity by inhibiting peptidoglycan biosynthesis makes murA, an attractive target for antibacterial agnets[22]

CONCLUSION

The antibiotic resistance becomes a serious issue and should be addressed by mitigating its effects and by providing alternative approaches for treating the deadly diseases due to the resistance mechanism. Thus the present study explores the biological pathways for the identification of novel antimicrobial targets which can be used to develop new antimicrobials that can combat the resistant mechanism of methicillin resistant *Staphylococcus aureus*.

REFERENCES

1. Powers JH, Antimicrobial drug development - the past, the present and the future. Clin Microbiol and Infect, 10 (4): 23-31, (2004).
2. Bhattacharyya BK and Sen SK, Antibiotics business: A glimpse. Indian J Biotechnol, 5: 471-176, (2006).
3. Fitzgerald JR, Sturdevant DE, Mackle SM, Gill SR and Musser JM, Evolutionary genomics of *Staphylococcus aureus*: Insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. PNAS, 98(15): 8821-8826, (2001).
4. Moellering RC, Why has MRSA become such a successful pathogen in adults?, Clinical updates in Infectious diseases, 13 (2): 1-6, (2010).
5. Lee DS, Burd H, Liu J, Almaas E, Wiest O, Barabasi AL, Oltvai ZN and Kapatral V, Comparative genome-scale metabolic reconstruction and flux balance analysis of multiple *Staphylococcus aureus* genomes identify novel antimicrobial drug targets. J Bacteriol, 191 (12): 4015-4024, (2009).
6. Amer FM, El-Behedy EM and Mohtady HA, New targets for antibacterial agents. Biotechnol. Mol. Biol. Rev, 3 (3): 46-57, (2008).

7. Talbot GH, Bradley J, Edwards JE, Gilbert D, Scheld M and Bartlett JG, Bad bug need drugs: An update on the development pipeline from the antimicrobial availability task force of the infectious disease society of America. CID, 42: 657-668, (2006).
8. Henriksen ST, Liu J, Estiu G, Oltvai ZN and Wiest O, Identification of novel bacterial histidine biosynthesis inhibitors using docking, ensemble rescoring, and whole-cell assays. Bioorg Med Chem, 18: 5148-5156, (2010).
9. Singh S, Malik BK and Sharma DK, Choke point analysis of metabolic pathways in *E.histolytica*: A computational approach fro drug target identification. Bioinformation, 2 (2): 68-72, (2007).
10. Mehta S and Tagore S, Functional module analysis in metabolomics: Chokes. Advances in Computational Research, 1 (1): 1-4, (2009).
11. Perumal D, Lim CS, Sakharkar KR and Sakharkar MK, 'Load points and 'choke points' as nodes for prioritizing drug targets in *Pseudomonas aeruginosa*. Current Bioinformatics, 4 (1): 48-53, (2009).
12. Rahman SA and Schomburg D, Observing local and global properties of metabolic pathways: 'load points' and 'choke points' in the metabolic networks. Bioinformatics, 22 (14): 1767-1774, (2006).
13. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S and Madden TL, NCBI BLAST: a better web interface. Nucleic Acids Research, 36: W5-W9, (2008).
14. Zhang R and Lin Y, DEG 5.0, A database of essential genes in both prokaryotes and eukaryotes. Nucleic acids research, 37: D455-D458, (2009).
15. Lu Z, Szafron D, Greiner R, Lu P, Wishart DS, Poulin B, Anvik J, Macdonell C and Eisner R, Predicting sub-cellular localization of proteins using machine-learned classifiers. Bioinformatics, 20 (4): 547-556, (2004).
16. Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R, Dao P, Sahinalp SC, Ester M, Foster LJ and Brinkman FSL, PSORTb 3.0: Improved protein Subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics, 26 (13): 1608-1615, (2010).
17. Arcuri HA, Zafalon G, Marucci EA, Bonalumi CE, Silveira N, Machado JM, Azevedo W and Palma MS, SKPDB: a structural database of shikimate pathway enzymes. BMC Bioinformatics, 11 (12), (2010).
18. Coggins JR, Abell C, Evans LB, Frederickson M, Robinson DA, Roszak AW and Lapthorn AP, Experiences with the shikimate-pathway enzymes as targets for rational drug design. Biochemical Society Transactions, 31 (3): 548-552, (2003).
19. Laub MT and Goulian M, Specificity in two-component signal transduction pathways. Annual review of Genetics, 41: 121-145, (2007).
20. Stephenson K and Hoch JA, Two-component and phopsphorelay signal-transduction systems as therapeutic targets. Current opinion in pharmacology, 2: 507-512, (2002).
21. Singh V, Shi W, Almo SC, Evans GB, Furneaux RH, Tyler PC, Zheng R and Schramm VL, Structure and inhibition of a quorum sensing target from *Streptococcus pneumonia.* Biochemistry, 45 (43): 12929-12941, (2006).
22. Molina-Lopez J, Sanschagrin F and Levesque RC, A peptide inhibitor of murA UDP-N-acetylglucosmaine enolpyruvyl transferase: The first committed step in peptidoglycan biosynthesis. Peptides, 27: 3115-3121, (2006).