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In silico identification of putative drug targets in methicillin resistant *Staphylococcus aureus*: a subtractive genomic approach

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ABSTRACT
Staphylococcus aureus is a gram positive, coagulase positive coccus in the family staphyloccocaceae. It is an opportunistic organism that has emerged as one of the predominant pathogens in community and healthcare-associated infections with limited and less effective options for treatment in the face of a rising trend in the emergence of resistant strains. This fact has necessitated the search for alternative targets for development of new drugs. In this present study, a subtractive genomic (proteome) approach was used to identify potential drug targets in methicillin resistant *Staphylococcus aureus* using strain 252 (MSRA252). The complete proteome of MSRA 252 obtained from Uniprot database was subjected to CD-hit suite for clustering; NCBI BlastP suite against the human proteome to exclude homologous proteins; and sequence homology with Database of Essential Genes(DEG) to determine the indispensability of the proteins for the bacteria survival. The essential proteins were further analyzed to predict the metabolic pathways they were involved in using KEGG automatic annotation server (KAAS) and their subcellular locations using, Uniprot and PsortB suite subsequently. The sequence sorting, segregation and formatting was carried out using UFS Sequence Analysis Application after each successive step. The study identified 291 essential non homologous proteins to human out of 2640. Further analysis with KAAS revealed that 114 (33 predicted membrane-associated) of the essential non homologous proteins were involved in different metabolic pathways in the organism and 60 of these were implicated in pathways unique to the bacteria relative to human (host). The study revealed a number of putative, essential non homologous protein candidates that could be further explored for the development of alternative treatments and vaccines for methicillin resistant *Staphylococcus aureus* infections.

Keywords: Subtractive genomics, BlastP, CD-hit, KAAS, MSRA 252.

1.0 INTRODUCTION
Staphylococcus aureus was first identified by Rosenbach in 1884 as a pathogen of wound infections and furunculosis. It is a common cause of skin and soft tissue infections worldwide and has also been implicated in conditions such as furuncle/carbuncles, cellulities, septic thrombophlebitis, bacteraemia, epidural abscess, osteomyelitis and prosthestic joint infection[1-2]. *Staphylococcus aureus* is a versatile and virulent pathogen. It has become a global health concern especially in health care setting because of the increasing high incidence, morbidity and antimicrobial resistance[3]. Approximately 20% of healthy persons are persistent carriers and 60% are intermittent carriers of *S.aureus*. Its resistance to methicillin dates back to 1961 soon after the introduction of the drug

and have till date become the most important antimicrobial resistant problem in *S. aureus* infections. Methicilin-resistant *Staphyloccus aureus* (MRSA) infections are now the aetiological pathogens for majority of the healthcare-associated infections with an estimated 1.5million cases of hospital-acquired MSRA infection annually worldwide [1]. It is responsible for significant morbidity and mortality as well as huge burden on the global budget. Options for management of *S. aureus* infections have been limited by the emergence of resistance strains. Vancomycin was considered to be a reference standard for the treatment of invasive MRSA infections, however, the use has been affected by factors such as limited tissue distribution as well as emergence of strains with reduced susceptibility and *in vitro* resistance. Other options that has been reported and their targets include Linozolid (50s ribosomes), Tigecycline (30S ribosomal subunit) Daptomycin (cell membrane components) Dalbavancin (cell wall synthesis) Telavancin (cell wall synthesis & membrane components) Oritavancin (transmembrane potential) Ceftobiprole and Cetaroline (penicillin Binding protein2a [peptidoglycan]) and Iclaprim (dihydrofolatereductase) [4]. The efficiency and the use of these treatment options leaves much to be desired and thus the need for novel and better targets for antibiotics against resistant strains of *S. aureus* is important.

Genomic–sequencing projects of pathogens and human is revolutionizing microbial target identification and drug development. Genomics and Bioinformatics provide new options in identifying optimal targets for drug development. The success of genomic projects in this era has delivered well over 500 complete microbial and human genome data to the science-world. Genomic approach to identifying potential drug targets in microorganism is based on two criteria, **essentiality** and **selectivity** of the genes/gene products (proteins). Based on this, subtractive genomic approach has been proposed for the prediction of potential targets of antimicrobial drugs[5-6]. This approach has been reported in literatures in successful identification of novel targets in *Streptococcus pneumonia* and *Haemophilus influenza* [7], *Mycobacterium tuberculosis* [8-9], *Bacillus anthracis* [10], *Salmonella typhi* [11], *Aeromonas hydropila* [12], *Neisseria gonorrhoeae* [13] and *Bacterial meningitis* [14].

In this present study, we utilized a step-wise subtractive genome (proteome) approach to identify potential drug targets for Methicillin-resistant *Staphylococcus aureus* (MSRA) using the complete, proteome of strain 252. The complete proteome of this strain is freely accessible at the Uniprot database.

2.0 MATERIALS AND METHODS

The subtractive genomic approach for this study was carried out in a stepwise manner which is summarized in figure 1 and sequentially reported below.

2.1 Sequence retrieval of pathogen proteins

The complete proteome of Methicillin-resistant *Staphylococcus aureus* strain 252 was retrieved from the Uniprot (*http://www.uniprot.org/taxonomy/complete-proteomes*) protein database in fasta format.

2.2 Proteome Clustering

The proteins of MRSA strain 252 retrieved from the Uniprot database were subjected to CD-hit suite using sequence identity cut off value at 70% [15]. Proteins that were more than 70% identical were considered to be duplicates or paralogs and were excluded from the resulting set. The non-paralog proteins were splitted into groups of 40 amino protein sequences each using UFS Sequence Analysis Application (http:*//www.ufumes.com/biotools*)- an application developed by this group, to organize retrieve and save sequences into fasta format. It can split proteomes into smaller sets; exclude defined sequences from a larger set using the Split and Negablast options respectively.

2.3 Identification of human non-homologs

Each group of non-paralog proteins were then subjected to NCBI BlastP suite in turn against Human (host) proteome using threshold expectation value of 10^{-4} to identify the human non-homologous proteins of MRSA[10,16]. The proteins that produced hits at this expectation value (10^{-4}) were considered human homologs and were excluded ('no good target') from the retrieved CSV file of the BlastP output results. The sequences of the non-homologs from each group were retrieved, unified and fasta-formatted using the UFS Sequence Analysis Application.

2.4 Similarity search for essential protein

The selected human non-homologous proteins were then subjected to sequence homology search against the Database of Essential Genes (DEG) to identify the proteins that are indispensable for the survival of the organism. A maximum threshold expectation value of 10^{-10} and a minimum bit-score cut-off of 100 were set as parameters to identify essential human non homologs [17].

2.5 Metabolic pathway analysis

The resulting human non-homologous essential proteins of MSRA obtained from DEG analysis were further subjected to metabolic pathway analysis by KEGG automatic annotation server (KAAS) (http://www.genome.jp/tools/kaas/) to identify their involvement in essential metabolic pathways [18]. KAAS provides functional annotation of genes through BLAST comparisons against the manually curated KEGG GENES database. The result contains KO (KEGG Orthology) assignments that specify the metabolic proteins. It also automatically generates KEGG pathways using the assigned KO.

2.6 Detection of proteins involved in unique pathways

The unique metabolic pathways of MRSA against Homo sapiens were identified manually by checking the metabolic pathways generated by KASS on the KEGG

(Kyoto Encyclopedia of Genes and Genomes) Genome Database [19]. Pathways common to both organisms were excluded and the unique pathways to MSRA relative to human were retained.

(PSL-pred), Sub-cellular localization of essential human non-homologous proteins was done by using Uniprot, CELLO v.2.5 [20] and then complimented by PSORTb, a System Vector Machine (SVM) based method, was used to predict the subcellular localization of the essential proteins [21].

2.7 Protein Sub-Cellular Localization Prediction

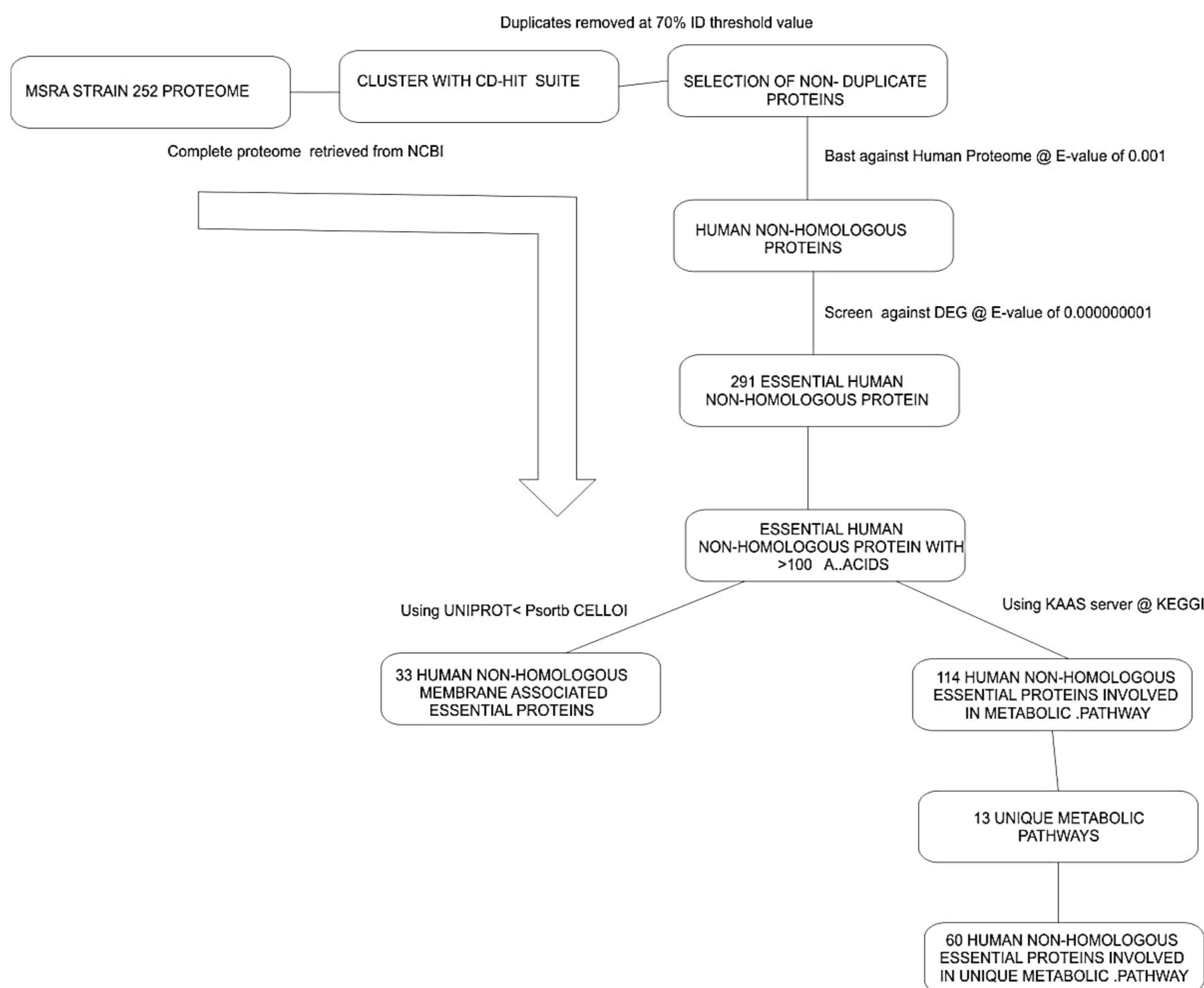

Figure 1: Schematic illustration of the methodology.

3.0 RESULTS AND DISCUSSION

Essential genes/gene products are required by pathogenic organism for survival. Identifying unique essential proteins in microorganism is pivotal in the development of novel drugs. We investigated the potential drug targets in methicillin resistant strains of *Staphylococcus aureus* using subtractive genome approach. The summary of the results of the successive analytical steps are presented in table 1 below.

Table 1: Step-wise subtractive analytical results for MSRA.

ANALYTICAL STEPS	TOTAL NUMBER OF PROTEINS
Retrieved Proteome	**2640**
Non-paralogs/ Non-duplicates returned by CD-HIT	**2576**
Paralogs/Duplicates (70% identical)	**64**
Human non-homologous proteins (E-value 10^{-4})	**1825**
Essential Proteins from DEG (E-value 10^{-10})	**291**
Essential Proteins in metabolic pathways	**114**
Proteins in unique metabolic Pathways	**60**
Human non-homologous membrane- associated essential proteins	**33**

Table 2: Result of identified human non-homologous essential proteins involved in metabolic pathways.

S/N0	ACESSION NUMBER	GENE	PROTEIN NAME	SUBCELLAR LOCATION	TYPE OF PATHWAY
1	Q6GE36	narG	Nitrate Reductase Alpha Chain	Extracellular component	U
2	Q6GE41	nreB	Oxygen Sensor Histidine Kinase NreB	Cytoplasm	U
3	Q6GEB1	SAR2408	PTS System,arbutin-like IIBC component	Inner Membrane	U
4	Q6GEE4	ureC	urease subunit alpha	Cytoplasm	U
5	Q6GEE5	ureB	urease subunit beta	Cytoplasm	U
6	Q6GEE6	ureA	urease subunit gamma	Cytoplasm	U
7	Q6GEH2	femX,fmhB	Lipid II:glycineglycyltranferase	Cytoplasm	U
8	Q6GEJ8	rplF	50S ribosomal protein L6	cellular Component	NU
9	Q6GEJ9	rplR	50S ribosomal protein L18	cellular Component	NU
10	Q6GEK1	rpmD	50S ribosomal Protein L30	cellular Component	NU
11	Q6GEK9	rpoA	DNA-directed RNA polymerase subunit alpha	cellular Component	NU
12	Q6GEN4	lacA	galactose-6-phosphate isomerase subunit LacA	Cytoplasm	NU
13	Q6GEN6	lacC	Tagatose-6-phosphate kinase	Cytoplasm	NU
14	Q6GER8	mtlD	Mannitol-1-phosphate 5-dehydrogenase	Cytoplasm	NU
15	Q6GER9	mttA,mtlF	Mannitol specific phosphotransferase enzyme IIA component	Cytoplasm	U
16	Q6GET2	SAR2231	Putative mannose-6-phosphate isomerase	Cytoplasm	U
17	Q6GEV0	fbaA	Fructose bisphosphatealdolase	Cytoplasm	U
18	Q6GEV4	rho	Transcription termination factor Rho	Cytoplasm	NU
19	Q6GEW8	atpF	ATP synthase subunit b	Inner membrane, Single-pass membrane protein	NU
20	Q6GEX5	murA1	UDP-N-acetylglucosamine 1-carboxyl transferase	Cytoplasm	U
21	Q6GEX6	fabZ	3-hydroxyacyl-[acyl-carrier-protein]dehydrataseFabZ	Cytoplasm	NU
22	Q6GEX8	SAR2185	Single-stranded DNA-binding protein	Cytoplasm	NU
23	Q6GEY5	yidC	Membrane protein insertaseYidC	cell membrane, Multi -pass membrane protein	U
24	Q6GEZ1	ddl	D-alanine--D-alanine ligase	Cytoplasm	U
25	Q6GEZ2	SAR2169	UDP-N-acetylmuramoyl-tripeptide --D-alanyl-D-alanine ligase	Cytoplasm	U
26	Q6GEZ6	kdpA1	Potassium transporting ATPase A Chain 1	inner Membrane, Multi - Pass Membrane Protein	U
27	Q6GF02	acpS	Holo-[acyl-carrier-protein] synthase	Cytoplasm	NU
28	Q6GFD7	ppaC	probable manganese- dependent inorganic pyrophosphatase	Cytoplasm	NU
29	Q6GFE4	nadE	NH(3) - dependentNAD(+) synthetase	Cytoplasm	NU
30	Q6GFF2	pcrA	ATP- dependent DNA Helicase PcrA	Cytoplasm	NU
31	Q6GFF3	ligA	DNA ligase	Cytoplasm	NU
32	Q6GFF6	gatC	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	Cytoplasm	NU
33	Q6GFG3	SAR1985	Putative exonuclase	Cytoplasm	NU
34	Q6GFH2	vraS	Sensor protein VraS	Inner Membrane, Multi - Pass Membrane Protein	U
35	Q6GFI3	mgt	Monofunctionalglycosyltransferase	Outer Membrane, Single - Pass Membrane protein	U
36	Q6GFW8	murC	UDP-N-acetylmuramate--L-alanine Ligase	Cytoplasm	U
37	Q6GFX8	SAR1807	putative transglycosylase	Inner Membrane	U
38	Q6GFY2	SAR1803	PTS system IIBC component	Inner Membrane	U
39	Q6GFZ6	ackA	Acetate Kinase	Cytoplasm	U
40	Q6GG04	dnaE	DNA polymerase III subunit alpha	Cytoplasm	NU
41	Q6GG13	phoP	alkaline phosphatase synthesis transcriptional regulatory protein	Cytoplasm	U
42	Q6GG14	phoR	Alkaline Phosphatase Sythesis Sensor Protein	Inner Membrane	U
43	Q6GGA7	nadD	probable nicotinate- nucleotide adenylyltransferase	Cytoplasm	NU
44	Q6GGB4	SAR1664	uncharacterised protein	cellular component	NU
45	Q6GGD7	dnaG	DNA primase	cellular component	NU
46	Q6GGE7	pbpF	Penicillin binding Protein PBP2B	Periplasmic	U
47	Q6GGH1	accB	Biotin Carboxy carrier protein of acetyl-CoA Carboxylase	cellular component	U
48	Q6GGK6	srrA	transcriptional Regulatory Protein SrrA	Cytoplasm	U
49	Q6GGK7	srrB	Sensor protein SrrB	Outer Membrane, Multi - Pass Membrane Protein	U
50	Q6GGT4	cmk	cytidylate kinase	Cytoplasm	NU
51	Q6GGV3	birA	BirAbifunctional protein [includes: biotin operon repressor biotin--[acetyl-CoA-carboxylase] synthetase	Cytoplas	NU
52	Q6GGV9	pbp2	penicillin binding Protein 2	Outer Membrane	U
53	Q6GGY5	crr	glucose specific phosphotranferase enzyme IIA component	Cytoplasm	U
54	Q6GH30	femB	aminoacyltranferaseFemB	Cytoplasm	U
55	Q6GH31	femA	aminoacyltranferaseFemA	Cytoplasm	U
56	Q6GH33	trpB	Tryptophan synthase beta chain	Cytoplasm	U

57	Q6GH45	mprF	phosphatidylglycerollysyltransferase	innner membrane, multi-pass membrane protein	NU
58	Q6GH52	plsY	Glycerol-3-phosphate acyltransferase	inner membrane, multi-pass membrane protein	NU
59	Q6GHH7	pyrH	uridylate kinase	Cytoplasm	NU
60	Q6GHK6	plsX	phosphate acyltransferase	Cytoplasm	NU
61	Q6GHP9	ftsZ	Cell Division Protein FtsZ	Cytoplasm	U
62	Q6GHQ0	ftsA	Cell Division Protein FtsA	Cytoplasm	U
63	Q6GHQ1	divlB	Cell Division Protein DivlB	Outer Membrane, Single - Pass Type II Membrane	U
64	Q6GHQ2	murD	UDP-N-acetylmuramoylalanine--D-glutamate ligase	Cytoplasm	U
65	Q6GHQ3	mraY	phospho-N-acetylmuramoyl-pentapeptidetransferase	Inner Membrane, Multi - Pass Membrane Protein	U
66	Q6GHQ4	pbpA	penicillin Binding protein 1	Periplasmic	U
67	Q6GHT5	murI	Glutamate racemase	Cytoplasm	NU
68	Q6GHT9	uvrC	UvrABC system protein C	Cytoplasm	NU
69	Q6GHT9	uvrC	UvrABC system protein C	Cytoplasm	NU
70	Q6GHW1	coaD	phosphopentetheineadenylyitransferase	Cytoplasm	NU
71	Q6GHX2	SAR1087	Putative Cell division Protein	Inner Membrane	U
72	Q6GHZ6	rnj1	Ribonuclease J 1	Cytoplasm	NU
73	Q6GI01	pstl	Phosphoenolpyruvate-protein phosphotransferase	Cytoplasm	U
74	Q6GI09	SAR1049	Putative cobalt transport protein	Inner membrane	NU
75	Q6GI66	murE	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-L-lysine ligase	Cytoplasm	U
76	Q6GIA4	fabH	3-oxoacyl-[acyl-carrier-protein] synthase 3	Cytoplasm	NU
77	Q6GIC4	spsA	iniactive signal peptidase	Periplasmic	NU
78	Q6GIF3	dltD	Putative lipoteichoic acid biosynthesis Protein	Periplasmic	NU
79	Q6GIF5	dltB	Putative activated D-alanine transport protein	Inner membrane	NU
80	Q6GIH7	SAR0872	Lipoprotein	Periplasmic	NU
81	Q6GIN8	secA	Protein translocase Subunit SecA	cell membrane, peripheral membrane protein, cytoplasmic side	U
82	Q6GIQ3	murB	UDP-N-acetylenolpyruvoylglucosaminereductase	Cytoplasm	U
83	Q6GIU2	fruA	PTS transport system,fructose specific IIABC component	Inner Membrane	U
84	Q6GIU3	SAR0752	Tagatose-6-phosphate kinase	Cytoplasm	NU
85	Q6GIV9	uppP	Undecaprenyl-diphosphatase	Inner Membrane, Multi - Pass Membrane Protein	U
86	Q6GJ11	graR	Response Regulatory Protein GraR	Cytoplasm	U
87	Q6GJ76	mvak2	phosphomevalonate kinase	cellular component	U
88	Q6GJ80	pta	phosphate acetyltransferase	Cytoplasm	U
89	Q6GJA3	folE2	GTP cyclohydrolase	Cytoplasm	NU
90	Q6GJC9	rplJ	50S ribosomal protein L10	cellular component	NU
91	Q6GJE0	cysE	serine acetyltranferase	Cytoplasm	U
92	Q6GJF5	folK	Putative hydroxymethyldihydropteridinepyrophosphokinase	2-amino-4-hydroxy-6-	NU
93	Q6GJF6	folB	Dihydroneopterinaldolase	Cytoplasm	NU
94	Q6GJF7	folP	Dihydropteroate synthase	Cytoplasm	NU
95	Q6GJG8	mfd	Transcriptoin-repair -coupling factor	Cytoplasm	NU
96	Q6GJH0	rplY	50S ribosomal protein L25	cellular component	NU
97	Q6GJI7	holB	Putative DNA polymerase III, delta' subunit	Cytoplasm	NU
98	Q6GJI9	tmk	Thymidylate	Cytoplasm	NU
99	Q6GK46	SAR0263	putative PTS transport system protein	Inner membrane	U
100	Q6GK62	SAR0247	Putative zinc-binding dehydrogenase	Cytoplasm	NU
101	Q6GK72	SAR0235	putative PTS transport system,IIBC component	Inner Membrane	U
102	Q6GKB7	ptsG	PTS system glucose-specific EIICBA component	Inner membrane, multi-pass membrane protein	U
103	Q6GKG6	deoB	Phosphoentomutase	Cytoplasm	NU
104	Q6GKN5	SAR0069	Sensor Kinase Protein	Inner Membrane	U
105	Q6GKN6	kdpE	response regulator protein	Cytoplasm	U
106	Q6GKQ7	mecA	penicillin binding protein 2 Prime	Outer membrane	U
107	Q6GKS7	walR,yycF	transcriptional Regulatory Protein WalR	Cytoplasm	U
108	Q6GKS9	DnaC	DnaB-like helicase	Cytoplasm	U
109	Q6GKT0	rplI	50S ribosomal protein L9	cellular component	NU
110	Q6GKU1	recF	DNA replication and repair protein RecF	Cytoplasm	NU
111	Q6GKU3	dnaN	DNA polymerase III subunit beta	Cytoplasm	NU
112	Q6GKU4	dnaA	Chromosomal Replication initiator Protein DnaA	Cytoplasm	U
113	Q6GIL5	gpml	2,3-bisphosphoglycerate - independent phosphoglyceratemutase	cellular component	U
114	Q6GEK3	secY	Protein translocase subunit SecY	Inner Membrane	U

Note: U =involved in a Unique metabolic pathway and NU= involved in a common metabolic pathway

Table 3: Unique pathways of MSRA when compared to *H. sapiens.*

Pathway Serial Number	Unique Metabolic Pathways	Number of Proteins Implicated
1	Microbial metabolism in diverse environments	4
2	Biosynthesis of secondary metabolites	4
3	Methane metabolism	4
4	Carbon fixation pathways in prokaryotes	2
5	D-Alanine metabolism	1
6	Peptidoglycan biosynthesis	14
7	Atrazine degradation	3
8	Tetracycline biosynthesis	2
9	Two-component system	12
10	Bacterial secretion system	10
11	Phosphotransferase system (PTS)	9
12	Cell cycle - Caulobacter	6
13	Beta-Lactam resistance	3

Table 4: Result of identified human non-homologous essential proteins involved in metabolic pathways unique to MSRA.

S/N	PROTEIN ACCESSION NUMBER	UNIQUE PATHWAY	S/N	PROTEIN ACCESSION NUMBER	UNIQUE PATHWAY
1	Q6GE41	9	31	Q6GIN8	11
2	Q6GEE4	7	32	Q6GIQ3	6
3	Q6GEE5	7	33	Q6GIV9	6
4	Q6GEE6	7	34	Q6GJ11	9
5	Q6GEH2	6	35	Q6GJ80	1,3,4
6	Q6GEK3	10	36	Q6GJE0	1
7	Q6GER9	11	37	Q6GKB7	11
8	Q6GEV0	1,2,3	38	Q6GKS7	9
9	Q6GEX5	6	39	Q6GKU4	12
10	Q6GEY5	10	40	Q6GE36	9
11	Q6GEZ1	5	41	Q6GEB1	11
12	Q6GEZ6	9	42	Q6GET2	2
13	Q6GFH2	9	43	Q6GEZ2	6
14	Q6GFI3	6	44	Q6GFX8	6
15	Q6GFW8	6	45	Q6GFY2	11
16	Q6GFZ6	1,3,4	46	Q6GG13	9
17	Q6GGK6	9	47	Q6GG14	9
18	Q6GGK7	9	48	Q6GGE7	6,13
19	Q6GGY5	10	49	Q6GGH1	8
20	Q6GH30	6	50	Q6GGV9	13
21	Q6GH31	6	51	Q6GHQ4	13
22	Q6GH33	2	52	Q6GHX2	12
23	Q6GHP9	12	53	Q6GIU2	11
24	Q6GHQ0	12	54	Q6GJ76	1
25	Q6GHQ1	12	55	Q6GK46	11
26	Q6GHQ2	6	56	Q6GK72	11
27	Q6GHQ3	6	57	Q6GKN5	9
28	Q6GI01	11	58	Q6GKN6	9
29	Q6GI66	6	59	Q6GKQ7	13
30	Q6GIL5	1,2,3	60	Q6GKS9	12

The retrieved complete proteome of MRSA 252 contained 2640 proteins which were clustered at 70% identity level to yield 2576 non-paralog proteins. The resulting non paralogs was blasted against the human (host) proteome using NCBI Blastp suite. This yielded 1825 non-homologous proteins. These are selective potential targets since they are not homologous to the host's. Screening the non-homologous proteins through DEG produced 291 essential proteins that are indispensable to the organism for survival. Further screening with KAAS identified 114 proteins involved in metabolic pathways and 60 of these were implicated in metabolic pathways unique to the organism relative to the host (table1). Thirty three of the proteins were predicted to be membrane-associated in all. These are likely target for vaccine development.

The 61 essential unique proteins were involved in 13 vital unique metabolic pathways (table 3). Fourteen (14) of the non-homologous essential were involved in peptidoglycan biosynthesis, twelve(12) in Two components regulatory system, eleven(11) in phosphotransferase system (PTS), six (6) in Cell cycle, one(1) in D-alanine metabolism as well as others.

Peptidoglycan biosynthesis is responsible for the integrity of the bacteria cell wall as well some virulence in host and has been the target of some antibiotics [22]. Two component regulatory system (TCS) play essential roles in signaling events such as cell-cell communication, adaptation to environment and pathogenesis and has also been proposed as target for developing antibiotics [23]. The phosphotransferases system is an essentially specialized system in bacteria built around specific transporters that import resources (sugar). It is an adaptation for the opportunistic lifestyle of bacteria [24-25]. The cell cycle (Caulobacter) and the D-alanine metabolism pathways

are also indispensable and peculiar to prokaryotes. The uniqueness of these pathways and their indispensability in bacteria such as S. *aureus* makes the accessory proteins strong targets for the development of variety of antibiotics. The inhibition of penicillin binding proteins implicated in beta-lactam resistance pathway as well as peptidoglycan biosynthesis has been suggested which could reduce the burden of resistance in the organism and thereby improving treatment options. The membrane associated essential human non homologous proteins are usually virulent factors and potential candidates for vaccines. They are usually easier to study using computational tools. Further work will be devoted to screening these proteins for B-cell, T-cell epitopes and binding capacity to human MHC molecules. Plant derived extracts can also be screened for inhibitory actions on these identified protein targets.

4.0 CONCLUSION

In conclusion, this *in silico* study using a subtractive genomic approach has identified 13 unique metabolic pathways and 60 unique essential proteins and 33 membranes – associated proteins as drug targets. Unique pathways are usually ideal for drug targets to avert toxicity and cross reactions as they are not present in the host. This approach has shown an overwhelming capacity to rapidly assess and identify these targets thereby reducing the laborious, capital-intensive and rigorous process of screening a whole organism for drug targets on whole genome scale in the laboratory.

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