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Potential therapeutic drug target identification in Community Acquired-Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) using computational analysis

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Abstract:
The emergence of multidrug-resistant strain of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) strain has highlighted the urgent need for the alternative and effective therapeutic approach to combat the menace of this nosocomial pathogen. In the present work novel potential therapeutic drug targets have been identified through the metabolic pathways analysis. All the gene products involved in different metabolic pathways of CA-MRSA in KEGG database were searched against the proteome of *Homo sapiens* using the BLASTp program and the threshold of E-value was set to as 0.001. After database searching, 152 putative targets were identified. Among all 152 putative targets, 39 genes encoding for putative targets were identified as the essential genes from the DEG database which are indispensable for the survival of CA-MRSA. After extensive literature review, 7 targets were identified as potential therapeutic drug target. These targets are Fructose-bisphosphate aldolase, Phosphoglyceromutase, Purine nucleoside phosphorylase, Uridylate kinase, Tryptophan synthase subunit beta, Acetate kinase and UDP-N-acetylglucosamine 1-carboxyvinyltransferase. Except Uridylate kinase all the identified targets were involved in more than one metabolic pathways of CA-MRSA which underlines the importance of drug targets. These potential therapeutic drug targets can be exploited for the discovery of novel inhibitors for CA-MRSA using the structure based drug design (SBDD) strategy.

Keywords: Drug target, metabolic pathways, CA-MRSA, KEGG, DEG

Background:
Methicillin resistant *Staphylococcus aureus*, or MRSA is a gram-positive bacterial pathogen which is resistant to methicillin and other beta-lactum antibiotics. It is a major causative agent of skin and soft-tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis **[1, 2,]**. The original MRSA infections associated with exposure in the health care setting, particularly in hospitals are referred to as hospital-acquired MRSA (HA-MRSA) **[3]**. In 1990s, a new strain of MRSA emerged in the community setting occurring among young healthy individuals with no exposure to the healthcare setting. The infections caused by these strains are called community-acquired MRSA (CA-MRSA) **[4, 5]**. Since then, this community-acquired MRSA strain (CA-MRSA) has quickly spread across the globe **[6-8]**. Outbreaks of CA-MRSA have been reported among children

[9], athletes **[10]**, nurseries **[11]** and obstetrical wards **[12]**. The CA-MRSA strains have been involved in skin and soft tissue infections including furuncles, abscesses, folliculitis, impetigo, cellulitis, and, more rarely, in cases of severe sepsis, necrotizing fascitis, and necrotizing pneumonia **[13]**. The CA-MRSA strain is commonly known as the *Staphylococcus aureus* subsp. *aureus MW2*. Popovich *et al*. reported that CA-MRSA may be replacing the traditional hospital-acquired MRSA (HA-MRSA) **[14].** The spread of resistant CA-MRSA strains across the globe becoming more common and posing potential threat to the life of community **[15]**. Because of multidrug resistance, particularly among CA-MRSA, alternative and effective therapeutic options are urgently needed. With the availability of complete genome sequences of CA-MRSA **[16]**, it has now paved the new way for identifying the novel drug targets. Through the complete genome analysis of the pathogen, it is possible to compile a list of potential gene products and their functions which are non-homologous to the proteome of *Homo sapiens*. In the present work novel potential theapeutic drug targets have been identified through the metabolic pathways analysis in the community acquired-methicillin resistant *Staphylococcus aureus*.

Methodology:

The entire genome of *Staphylococcus aureus* subsp. *aureus MW2* (CA-MRSA), was sequenced in the year 2002. It is available on the website http://www.genome.jp (Accesion No. NC_003923) which contain 2820462 base pairs and 2624 protein encoding genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database **[17]** was used http://www.kegg.jp/kegg/pathway.htm for the retrieval of metabolic pathways for the community-acquired methicillin resistant *Staphylococcus aureus* (Entry no. T00086). The metabolic pathway of CA-MRSA was analyzed which was containing 76 different types of metabolic pathways. All enzymes involved in the different metabolic pathways were listed in a table. The most important criteria for selecting any enzyme or protein as a potential drug target in a pathogen is that it should be non-homologous to the host i.e. *Homo sapiens*. The gene products involved in different metabolic pathways of CA-MRSA genome were subjected to the database searching against the proteome of the *Homo sapiens* using the BLASTp program **[18]**. The threshold of E-value (expect value) was set to as 0.001. The similar protein sequences which were having less than 30% identity or less than 80% query coverage to the *Homo sapiens* proteome were considered as the non-homologous to the human. Those enzymes can be considered as the unique potential therapeutic drug targets for the drug designing. After performing the database searching of all metabolic enzymes (gene products) of CA-MRSA against the human proteome, 220 targets were identified as non-homologous to *Homo sapiens*. These enzymes were involved in 50 different metabolic pathways. Further analysis for all 220 targets was carried out and it was found that some duplicate targets were involved in more than one metabolic pathway. The list of all putative targets was further refined and duplicates were removed. Finally 152 targets were identified as unique putative drug targets. After identifying the novel potential drug targets from metabolic pathways of CA-MRSA, the genes coding for the important enzymes were further searched in the DEG 6.8 database **[19]** to identify the essentiality or non-essentiality of the genes for the survival of the pathogen. DEG provides the database of essential genes which are indispensable for the survival of an organism (http://www.essentialgene.org/). DEG database has been classified in to two categories prokaryotes and eukaryotes. In the pathogens, essential gene products provide unique potential drug targets for antimicrobial targets. Among all 152 putative drug targets, 39 genes which encode for potential drug targets were identified as essential for the survival of the CA-MRSA.

Discussion:

Community acquired-methicillin resistant *Staphylococcus aureus* (CA-MRSA) strains are now becoming nosocomial pathogen to the human race. In comparison to hospital acquired MRSA, these strains cause infections suddenly, quickly, and with great severity in patients which leads to worse clinical outcome. CA-MRSA strains are more virulent than other strains and have very bad impact on conventional therapy particularly with beta-lactum antibiotics which are becoming ineffective for a variety of common staphylococcal infections especially for skin & soft tissue infections **[20]**. Therefore, we have to find the alternative approach to combat the menace of drug resistance of CA-MRSA. In the present work, post genomic approach has been applied for the identification of potential drug targets for the CA-MRSA. The genes involved in different metabolic pathways of CA-MRSA were analyzed and it was found that total 76 pathways were present in KEGG pathway database. KEGG is the largest database resource consisting 17 different types of databases. For identifying the putative drug targets in the genome of any pathogen, it should be present in the organism and posse's crucial functional role but absent in the *Homo sapiens*. Using the BLASTp program, database searching was performed for all the gene products involved in different metabolic pathways of CA-MRSA against the proteome of *Homo sapiens*. The threshold of E-value was given 0.001 which measures the significance of similarity to the host. Apart from the E-value threshold, the % identity and % query coverage was also considered as the parameter for identifying the putative drug targets non-homologous to the proteome of *Homo sapiens*. The protein sequences which were having more than 0.001 E-value and less than 25% sequence identity and/or less than 80% query coverage, were considered as non-homologous drug targets. Total 220 putative drug targets were identified **Table 1 (see supplementary material)**. Out of 220 targets, it was found that some targets (proteins) were involved in more than one metabolic pathway. All the duplicate targets were removed from the list and total 152 unique putative drug targets were identified. The genes encoded for 152 unique putative targets were again searched against the DEG (Database of Essential Genes) database to identify the essentiality of the genes for the survival of CA-MRSA. DEG is the database of essential genes which are indispensable for the survival of any organism. After searching all 152 putative targets, 39 genes were identified as the essential for the survival of CA-MRSA **Table 2 (see supplementary material)**. All 39 essential gene products (targets) were analyzed and it was found that 20 putative drug targets were involved in more than one metabolic pathway. These 20 putative targets can be used as potential therapeutic drug targets for CA-MRSA. Out of 20 putative drug targets, it has been reported in literatures that 7 targets may be used as potential therapeutic drug targets. These targets are Fructose-bisphosphate aldolase (EC: 4.1.2.13), Phosphoglyceromutase (EC: 5.4.2.1), Purine nucleoside phosphorylase (EC: 2.4.2.1), Uridylate kinase (EC: 2.7.4.22), Tryptophan synthase subunit

beta (EC:4.2.1.20), Acetate kinase (EC:2.7.2.1) and UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7).

Fructose-bisphosphate aldolase (EC: 4.1.2.13)

Fructose-bisphosphate aldolase (FBA) enzyme is encoded by **fbaA** gene (ID: MW2049). This gene has been found essential in DEG database (DEG10020239) for *Staphylococcus aureus N315, Bacillus subtilis, Mycoplasma pulmonis and Escherichia coli*. FBA has been reported as potential therapeutic drug target in *Mycobacterium tuberculosis* and *Candida albicans* **[21, 22]**. These FBAs are involved in second reversible step of the glycolytic pathway, which supplies glyceraldehyde 3-phosphate for downstream enzymes in the pathway and fructose 1, 6-bisphosphate (FBP) for gluconeogenesis. Together, the substrates and products of the FBA reaction are crucial for the supply of these precursor molecules to other biochemical pathways essential for the survival of CA-MRSA. This enzyme is also involved in three other metabolic pathways i.e. pentose phosphate pathway, fructose and mannose metabolism & methane metabolism.

Phosphoglyceromutase (EC: 5.4.2.1)

Phosphoglyceromutase (PGM) enzyme is encoded by **pgm** gene (ID: MW0737). This gene has been found essential in DEG database (DEG10020010) for *Bacillus subtilis, Mycoplasma pulmonis, Mycoplasma genitalium* and *Salmonella enterica*. PGM interconvert 2-phosphoglycerate and 3-phosphoglycerate in the glycolytic and gluconeogenic pathways. This enzyme is also involved in glycine, serine, threonine metabolism, and methane metabolism in CA-MRSA. PGM has been reported as important drug target in *Wolbachia* endosymbiont from the filarial nematode, *Brugia malayi* (wBm) **[23]**.

Purine nucleoside phosphorylase (EC: 2.4.2.1)

Purine nucleoside phosphorylase (PNP) enzyme is encoded by **pnp** gene (ID: MW0110). This gene has been found essential in DEG database (DEG10020139) for *Staphylococcus aureus N315, E. coli* and *Acinetobacter baylyi*. PNP plays a crucial role in the phosphorolysis of purine nucleosides and deoxynucleosides to generate purine bases. This enzyme is also involved in pyrimidine, nicotinate and nicotinamide metabolism. PNP has been reported as potential therapeutic drug target in *M. tuberculosis* and *Streptococcus mutans* **[24, 25]**.

Uridylate kinase (EC: 2.7.4.22)

Uridylate kinase or UMP kinase (UMPK) enzyme is encoded by **pyrH** gene (ID: MW1141). This gene has been found essential in DEG database (DEG10170157) for *Staphylococcus aureus NCTC8325, M. tuberculosis, Mycoplasma pulmonis, Streptococcus pneumoniae, Pseudomona aeruginosa, Salmonella typhimurium, V. cholerae* etc. UMP kinase catalyses the phosphorylation of UMP by ATP to yield UDP which is involved in cell wall and RNA biosynthesis. UMPK is conserved in almost all prokaryotic organisms and has been reported as potential therapeutic drug target in *Staphylococcus aureus, Streptococcus pneumoniae* **[26, 27]**.

Tryptophan synthase subunit beta (EC: 4.2.1.20)

Tryptophan synthase subunit beta (TrpB) enzyme is encoded by **trpB** gene (ID: MW1259). This gene has been found essential in DEG database (DEG10020152) for *Staphylococcus aureus N315, M. tuberculosis, Streptococcus pneumonia, Haemophilus influenzae* and *Acenetobacter baylayi*. TrpB enzyme catalyzes the last step of the tryptophan biosynthetic pathway which is commonly present in almost all prokaryotic organisms but absent in mammals. This enzyme is also involved in the biosynthesis of phenylalanine and tyrosine as well as in the metabolism of glycine, serine and threonine amino acids. TrpB has been reported as potential therapeutic drug target in *Mycobacterium tuberculosis* and *Salmonella typhimurium* **[28, 29]**.

Acetate kinase (EC: 2.7.2.1)

Acetate kinase (ACK) enzyme is encoded by **ackA** gene (ID: MW1654). This gene has been found essential in DEG database (DEG10020202) for *Staphylococcus aureus N315, Mycoplasma pulmonis, Mycoplasma genitalium* and *E. coli*. ACK enzyme is involved in the formation of acetate from acetyl-CoA as a metabolic end product. It is involved in many metabolic pathways of CA-MRSA e.g. Taurine & hypotaurine, pyruvate, propanoate and methane metabolism. This enzyme is present in prokaryotic organisms and some eukaryotic organisms e.g. parasites but absent in mammals and it has been reported as attractive drug target for the development of anti-parasitic drugs **[30]**.

UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC: 2.5.1.7)

UDP-N-acetylglucosamine 1-carboxyvinyltransferase enzyme is encoded by **murA** gene (ID: MW2024). This gene has been found essential in DEG database (DEG10020231) for *Staphylococcus aureus N315, Mycobacterium tuberculosis, Bacillus subtilis, Salmonella enterica, Francisella novicida, Helicobacter pylori, E. coli* and *Acenetobacter baylayi*. MurA enzyme catalyses the biosynthesis of peptidoglycan polymer, consisting of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). Peptidoglycan is an integral constituent of bacterial cell wall which is indispensable for the survival of bacteria. UDP-N-acetylglucosamine 1-carboxyvinyltransferase (murA) enzyme catalyses the transfer of the enolpyruvyl group of phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of uridine diphospho-N-acetylglucosamine (UNAG). Furthermore, this enzyme is also involved in amino sugar & nucleotide sugar metabolism. MurA is essential enzyme present in all prokaryotic organism but absent in mammals. It has been reported as potential therapeutic drug target in *Haemophilus influenzae, Escherichia coli* and *Streptococcus pneumonia* **[31, 32, 33]**. Furthermore, except uridylate kinase all above potential therapeutic targets were involved in more than one metabolic pathways of CA-MRSA which underlines the importance of these targets. These drug targets can be used for the discovery of novel drugs which might potentially inhibit the growth of CA-MRSA.

Conclusion:

The metabolic pathway of nosocomial community acquired-methicillin resistant *Staphylococcus aureus* (CA-MRSA) strain was analyzed from the KEGG database. All the gene products involved in different metabolic pathways of CA-MRSA were searched against the proteome of *Homo sapiens* and 152 putative targets were identified. 39 genes encoding for important targets were identified as the essential from the DEG database which are indispensable for the survival of CA-MRSA. After extensive literature review, 7 targets were identified as potential therapeutic drug target. These targets are Fructose-bisphosphate aldolase (EC: 4.1.2.13), Phosphoglyceromutase

(EC: 5.4.2.1), Purine nucleoside phosphorylase (EC: 2.4.2.1), Uridylate kinase (EC: 2.7.4.22), Tryptophan synthase subunit beta (EC:4.2.1.20), Acetate kinase (EC:2.7.2.1) and UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7). Almost all these putative targets were involved in more than one metabolic pathways of CA-MRSA. These potential therapeutic drug targets can be exploited for the discovery of novel inhibitors for CA-MRSA using the structure based drug design (SBDD) strategy.

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Supplementary material:

Table 1: Metabolic Enzymes involved in different metabolic Pathways of CA-MRSA

Sr. No.	Accession No.	Pathways/ Putative Targets	E-value	% Identity
		Glycolysis / Gluconeogenesis		
1.	MW2435	Fructose-bisphosphatase [EC:3.1.3.11]	0.59	29
2.	MW2049	Fructose-bisphosphate aldolase (EC:4.1.2.13)	0.15	26
3.	MW0737	Phosphoglyceromutase (EC:5.4.2.1)	0.48	33
4.	MW1729	Phosphoenolpyruvate carboxykinase (EC:4.1.1.49)	2.7	29
5.	MW1312	PTS system glucose-specific enzyme II A component	5.9	35
6.	MW2244	PTS system arbutin-like IIBC component	0	0
		TCA Cycle		
7.	MW1173	2-oxoglutarate ferredoxin oxidoreductase subunit beta	0.81	25
8.	MW1030	Succinate dehydrogenase cytochrome b-558	1.1	33
9.	MW1729	Phosphoenolpyruvate carboxykinase (EC:4.1.1.49)	2.7	29
		Pentose phosphate pathway		
10.	MW0844	Glucose-6-phosphate isomerase (EC:5.3.1.9)	5e-08	23
11.	MW1721	Putative translaldolase (EC:2.2.1.2)	0.16	38
12.	MW0113	Phosphopentomutase (EC:5.4.2.7)	2.9	45
13.	MW2049	Fructose-bisphosphate aldolase	0.15	26
14.	MW2435	Fructose-bisphosphatase	0.59	29
		Pentose and glucuronate interconversions		
15.	MW2419	UTP-glucose-1-phosphate uridyltransferase	1e-10	23
		Fructose and mannose metabolism		
16.	MW2435	Fructose-bisphosphatase	0.59	29
17.	MW2085	Mannitol-1-phosphate 5-dehydrogenase	0.27	27
18.	MW2049	Fructose-bisphosphate aldolase (EC:4.1.2.13)	0.15	26
19.	MW0662	Fructose specific permease	No hits	0
20.	MW2082	PTS system mannitol specific IIBC component	No hits	0
		Galactose metabolism		
21.	MW2419	UTP-glucose-1-phosphate uridyltransferase	1e-10	23
22.	MW0223	PTS galactitol-specific enzyme IIC component	No hits	0
23.	MW2119	Tagatose-6-phosphate kinase	0.37	26
24.	MW2118	Tagatose 1,6-diphosphate aldolase	2.7	25
25.	MW2116	PTS system lactose-specific IIBC component	0.48	24
26.	MW1965	Sucrose-6-phosphate hydrolase	8.6	29
		Ascorbate and aldarate metabolism		
27.	MW0306	PTS system ascorbate-specific transporter subunit IIC	No hits	0
		Fatty acid biosynthesis		
28.	MW0865	3-oxoacyl-(acyl carrier protein) synthase III (EC:2.3.1.41)	0.81	30
		Ubiquinone and other terpenoid-quinone biosynthesis		
29.	MW0927	Menaquinone biosynthesis protein	2e-04	24
30.	MW1734	O-succinylbenzoic acid synthetase	4.1	30
		Oxidative phosphorylation		
31.	MW1030	Succinate dehydrogenase cytochrome b-558	1.1	33
32.	MW2033	F0F1 ATP synthase subunit A	0.6	27
33.	MW1860	Putative manganese-dependent inorganic pyrophosphatase (EC:3.6.1.1)	0.19	28
		Purine metabolism		
34.	MW0113	Phosphopentomutase (EC:5.4.2.7)	2.9	45
35.	MW0952	Phosphoribosylformylglycinamidine synthase II	2.00E-017	23
36.	MW0948	Phosphoribosylaminoimidazole carboxylase ATPase subunit	1.00E-004	22
37.	MW0110	Purine nucleoside phosphorylase (EC:2.4.2.1)	1.7	25
38.	MW2062	Purine nucleoside phosphorylase (EC:2.4.2.1)	0.028	23
39.	MW2537	Anaerobic ribonucleoside triphosphate reductase (EC:1.17.4.2)	0.12	26
40.	MW2143	DNA-directed RNA polymerase subunit alpha (EC:2.7.7.6)	0.007	22
41.	MW1646	DNA polymerase III alpha subunit	3.2	30
42.	MW1147	DNA polymerase III PolC	0.015	24
43.	MW0002	DNA polymerase III subunit beta (EC:2.7.7.7)	0.01	26
44.	MW1538	DNA polymerase III subunit delta	1.2	46
45.	MW0439	DNA polymerase III delta prime subunit	0.024	25
46.	MW0887	GTP pyrophosphokinase	4.2	30
47.	MW1051	Carbamate kinase (EC:2.7.2.2)	0.12	39
48.	MW2553	Carbamate kinase (EC:2.7.2.2)	0.061	25
		Pyrimidine metabolism		
49.	MW1088	Orotate phosphoribosyltransferase	2.00E-012	22
50.	MW1141	Uridylate kinase	1.7	26
51.	MW2143	DNA-directed RNA polymerase subunit alpha (EC:2.7.7.6)	0.007	22
52.	MW1646	DNA polymerase III alpha subunit	3.2	30
53.	MW1147	DNA polymerase III PolC	0.015	24
54.	MW0002	DNA polymerase III subunit beta (EC:2.7.7.7)	0.01	26
55.	MW1538	DNA polymerase III subunit delta	1.2	46
56.	MW0439	DNA polymerase III delta prime subunit	0.024	25
57.	MW2537	Anaerobic ribonucleoside triphosphate reductase (EC:1.17.4.2)	0.12	26
58.	MW0110	Purine nucleoside phosphorylase (EC:2.4.2.1)	1.7	25
59.	MW2062	Purine nucleoside phosphorylase (EC:2.4.2.1)	0.028	23
60.	MW0437	Thymidylate kinase	0.041	25
		Alanine, aspartate and glutamate metabolism		

61.	MW0426	Glutamate synthase large subunit	0.5	24
62.	MW0427	Glutamate synthase subunit beta (EC:1.4.1.13)	3.00E-012	22
63.		**Glycine, serine and threonine metabolism**		
64.	MW1281	Aspartate kinase	3.7	25
65.	MW1214	Aspartate kinase (EC:2.7.2.4)	4.3	30
66.	MW1215	Homoserine dehydrogenase	6.5	23
67.	MW1217	Homoserine kinase	2	26
68.	MW0737	Phosphoglyceromutase (EC:5.4.2.1)	0.48	33
69.	MW1260	Tryptophan synthase subunit alpha (EC:4.2.1.20)	0.39	22
70.	MW1259	Tryptophan synthase subunit beta (EC:4.2.1.20)	1.8	30
71.	MW0332	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase (EC:2.1.1.14)	1.2	44
72.	MW1550	5'-methylthioadenosine nucleosidase/S-adenosylhomocysteine nucleosidase	3.5	41
		Valine, leucine and isoleucine biosynthesis		
73.	MW1980	Ketol-acid reductoisomerase (EC:1.1.1.86)	0.039	35
74.	MW1977	Dihydroxy-acid dehydratase (EC:4.2.1.9)	0.29	33
75.	MW1981	d-Alanine metabolism	0.005	23
		Lysine biosynthesis		
76.	MW1215	Homoserine dehydrogenase	6.5	23
77.	MW1281	Aspartate kinase	3.7	25
78.	MW1214	Aspartate kinase (EC:2.7.2.4)	4.3	30
79.	MW1284	Dihydrodipicolinate reductase	4.1	40
80.	MW1943	Succinyl-diaminopimelate desuccinylase	4.00E-008	23
81.	MW1288	Diaminopimelate decarboxylase	1.00E-018	23
82.	MW1285	Tetrahydrodipicolinate acetyltransferase	6.7	34
83.	MW2005	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase	1.4,	59
		Lysine degradation		
84.	MW1693	D-alanine aminotransferase	0.74	43
		Beta-Lactam resistance		
85.	MW2608	Drp35	2.00E-004	22
86.	MW0032	Truncated methicillin resistance protein MecR1	0.55	23
87.	MW0031	Penicillin binding protein 2 prime	No hits	0
		Arginine and proline metabolism		
88.	MW1693	D-alanine aminotransferase	0.74	43
89.	MW2556	Arginine deiminase (EC:3.5.3.6)	2.7	34
90.	MW1051	Carbamate kinase (EC:2.7.2.2)	0.12	39
91.	MW2553	Carbamate kinase (EC:2.7.2.2)	0.061	25
92.	MW0157	Bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein (EC:2.3.1.35 2.3.1.1)	1.9	25
93.	MW0158	N-acetyl-gamma-glutamyl-phosphate reductase (EC:1.2.1.38)	4.6	38
		Histidine metabolism		
94.	MW2598	ATP phosphoribosyltransferase catalytic subunit (EC:2.4.2.17)	2.2	41
95.	MW2591	Bifunctional phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphatase protein	2.1	52
96.	MW2593	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase	4.3	34
97.	MW2592	Imidazole glycerol phosphate synthase subunit HisF	1.2	29
98.	MW0686	Histidinol-phosphate aminotransferase	1.00E-005	21
99.	MW2597	Histidinol dehydrogenase	0.23	25
		Tyrosine metabolism		
100.	MW0686	Histidinol-phosphate aminotransferase	1.00E-005	21
		Phenylalanine metabolism		
101.	MW0686	Histidinol-phosphate aminotransferase	1.00E-005	21
102.	MW1693	D-alanine aminotransferase	0.74	43
		Phenylalanine, tyrosine and tryptophan biosynthesis		
103.	MW1680	Bifunctional 3-deoxy-7-phosphoheptulonate synthase/chorismate mutase	1.3	24
104.	MW1355	3-dehydroquinate synthase	0.75	42
105.	MW0782	3-dehydroquinate dehydratase	0.28	28
106.	MW1547	Shikimate 5-dehydrogenase	0.038	23
107.	MW1354	3-phosphoshikimate 1-carboxyvinyltransferase (EC:2.5.1.19)	0.71	40
108.	MW1356	Chorismate synthase (EC:4.2.3.5)	3.2	30
109.	MW1254	Anthranilate synthase component I	1.6	22
110.	MW1256	Anthranilate phosphoribosyltransferase	0.96	25
111.	MW1258	N-(5'-phosphoribosyl)anthranilate isomerase	No hits	0
112.	MW1257	Indole-3-glycerol-phosphate synthase	1	31
113.	MW1260	Tryptophan synthase subunit alpha (EC:4.2.1.20)	0.39	22
114.	MW1259	Tryptophan synthase subunit beta (EC:4.2.1.20)	1.8	30
115.	MW1252	Prephenate dehydrogenase (EC:1.3.1.12)	1.7	30
116.	MW0686	Histidinol-phosphate aminotransferase	1.00E-005	21
		Novobiocin biosynthesis		
117.	MW1252	Prephenate dehydrogenase (EC:1.3.1.12)	1.7	30
118.	MW0686	Histidinol-phosphate aminotransferase	1.00E-005	21
		Beta-Alanine metabolism		
119.	MW2517	Pantoate--beta-alanine ligase (EC:6.3.2.1)	1.6	33
		Taurine and hypotaurine metabolism		
120.	MW0543	Phosphotransacetylase	0.85	29
121.	MW1654	Acetate kinase (EC:2.7.2.1)	9.1	41
		Selenocompound metabolism		
122.	MW0332	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase (EC:2.1.1.14)	1.2	44
		D-Glutamine and D-glutamate metabolism		
123.	MW1033	Glutamate racemase	0.091	24
124.	MW1066	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase (EC:6.3.2.9)	3.4	27
125.	MW1683	UDP-N-acetylmuramate--L-alanine ligase (EC:6.3.2.8)	0.33	22
		D-Arginine and D-ornithine metabolism		

126.	MW1693	D-alanine aminotransferase	0.74	43
		D-Alanine metabolism		
127.	MW1994	Alanine racemase	0.6	23
128.	MW2006	D-alanyl-alanine synthetase A (EC:6.3.2.4)	0.023	23
129.	MW1693	D-alanine aminotransferase	0.74	43
		Starch and sucrose metabolism		
130.	MW2299	PTS system sucrose-specific IIBC component	1	29
131.	MW1965	Sucrose-6-phosphate hydrolase	8.6	29
132.	MW0428	PTS enzyme II	0.62	38
133.	MW2419	UTP-glucose-1-phosphate uridyltransferase	1.00E-010	21
		Amino sugar and nucleotide sugar metabolism		
134.	MW0165	N-acetylmuramic acid-6-phosphate etherase	3.00E-008	20
135.	MW1668	PTS system N-acetylglucosamine-specific IIABC component	0.5	22
136.	MW0454	Bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	6.00E-015	21
137.	MW0130	Capsular polysaccharide synthesis enzyme Cap8G	0.055	27
138.	MW0139	Capsular polysaccharide synthesis enzyme Cap8P	1.00E-007	21
139.	MW2035	UDP-GlcNAc 2-epimerase	2.00E-007	22
139.	MW0295	N-acetylmannosamine-6-phosphate 2-epimerase (EC:5.1.3.9)	2.5	30
140.	MW0138	Capsular polysaccharide synthesis enzyme Cap8O	4.00E-013	23
141.	MW2024	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7)	0.78	26
142.	MW2048	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7)	0.32	25
143.	MW0700	UDP-N-acetylenolpyruvoylglucosamine reductase	1.5	24
144.	MW2459	PTS system glucose-specific IIABC component	3.5	38
145.	MW0844	Glucose-6-phosphate isomerase (EC:5.3.1.9)	5.00E-008	23
146.	MW2419	UTP-glucose-1-phosphate uridyltransferase	1.00E-010	23
		Peptidoglycan biosynthesis		
147.	MW2024	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7)	0.78	26
148.	MW2048	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC:2.5.1.7)	0.32	25
149.	MW0700	UDP-N-acetylenolpyruvoylglucosamine reductase	1.5	24
150.	MW1683	UDP-N-acetylmuramate--L-alanine ligase (EC:6.3.2.8)	0.33	22
151.	MW1066	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase (EC:6.3.2.9)	3.4	27
152.	MW2006	D-alanyl-alanine synthetase A (EC:6.3.2.4)	0.023	23
153.	MW2005	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase	1.4	59
154.	MW0645	Undecaprenyl pyrophosphate phosphatase (EC:3.6.1.27)	No hits	0
155.	MW1065	Phospho-N-acetylmuramoyl-pentapeptide-transferase	1.2	36
156.	MW1340	PBP2	2.7	25
157.	MW0604	Penicillin binding protein 4	1.3	23
158.	MW0899	UDP-N-acetylmuramoylalanyl-D-glutamate--L-lysine ligase	5	28
159.	MW2180	FmhB protein	0.1	29
160.	MW1261	Factor essential for expression of methicillin resistance	1.1	33
161.	MW1262	FemB protein	0.17	23
162.	MW1672	Transglycosylase	No hits	0
163.	MW1814	Glycosyltransferase	21	3
164.	MW1064	Penicillin-binding protein 1	2.5	21
165.	MW1504	Penicillin-binding protein 3	0.95	28
		Glycerolipid metabolism		
166.	MW1112	Putative glycerol-3-phosphate acyltransferase PlsX	9.6	23
167.	MW0297	Glycerol ester hydrolase	3	23
168.	MW2590	Triacylglycerol lipase precursor (EC:3.1.1.3)	4.8	32
169.	MW0898	Diacylglycerol glucosyltransferase	0.73	31
		Inositol phosphate metabolism		
170.	MW1940	Truncated beta-hemplysin	0.03	25
		Glycerophospholipid metabolism		
171.	MW1112	Putative glycerol-3-phosphate acyltransferase PlsX	9.6	23
172.	MW1940	Truncated beta-hemplysin	0.03	25
		Pyruvate metabolism		
173.	MW0201	Formate acetyltransferase	1.4	21
174.	MW1654	Acetate kinase (EC:2.7.2.1)	9.1	41
175.	MW0543	Phosphotransacetylase	0.85	29
176.	MW2286	Malate:quinone oxidoreductase (EC:1.1.5.4)	0.6	31
177.	MW2526	Malate:quinone oxidoreductase (EC:1.1.5.4)	0.25	21
178.	MW1729	Phosphoenolpyruvate carboxykinase (EC:4.1.1.49)	2.7	29
179.	MW1981	2-isopropylmalate synthase (EC:2.3.3.13)	0.005	23
		Propanoate metabolism		
180.	MW1654	Acetate kinase (EC:2.7.2.1)	9.1	41
181.	MW0543	Phosphotransacetylase	0.85	29
182.	MW0201	Formate acetyltransferase	1.4,	21
		Butanoate metabolism		
183.	MW1030	Succinate dehydrogenase cytochrome b-558	1.1	33
184.	MW0201	Formate acetyltransferase	1.4	21
		Methane metabolism		
185.	MW2049	Fructose-bisphosphate aldolase (EC:4.1.2.13)	0.15	26
186.	MW1654	Acetate kinase (EC:2.7.2.1)	9.1	41
187.	MW0543	Phosphotransacetylase	0.85	29
188.	MW0737	Phosphoglyceromutase (EC:5.4.2.1)	0.48	33
		Thiamine metabolism		
189.	MW1658	Thiamine biosynthesis protein ThiI	0.52	26
190.	MW2015	Hydroxyethylthiazole kinase (EC:2.7.1.50)	1.2	31
191.	MW2014	Thiamine-phosphate pyrophosphorylase	0.18	35
		Riboflavin metabolism		

192.	MW1709	Riboflavin biosynthesis protein	2.8	27
193.	MW1711	Riboflavin specific deaminase	0.003	24
194.	MW1710	Riboflavin synthase subunit alpha (EC:2.5.1.9)	0.21	30
		Vitamin B6 metabolism		
195.	MW0535	Phosphomethylpyrimidine kinase	0.037	25
196.	MW0474	Pyridoxal biosynthesis lyase PdxS	0.58	37
		Nicotinate and nicotinamide metabolism		
197.	MW0110	Purine nucleoside phosphorylase (EC:2.4.2.1)	1.7	25
198.	MW2062	Purine nucleoside phosphorylase (EC:2.4.2.1)	0.028	23
		Pantothenate and CoA biosynthesis		
199.	MW1980	Ketol-acid reductoisomerase (EC:1.1.1.86)	0.039	35
200.	MW1977	Dihydroxy-acid dehydratase (EC:4.2.1.9)	0.29	33
201.	MW2518	3-methyl-2-oxobutanoate hydroxymethyltransferase (EC:2.1.2.11)	3.2	33
202.	MW2367	2-dehydropantoate 2-reductase (EC:1.1.1.169)	2.2	26
203.	MW2519	2-dehydropantoate 2-reductase (EC:1.1.1.169)	0.49	32
204.	MW2517	Pantoate-beta-alanine ligase (EC:6.3.2.1)	1.6	33
		Biotin metabolism		
205.	MW2346	6-carboxyhexanoate--CoA ligase (EC:6.2.1.14)	0.75	26
206.	MW2350	Dethiobiotin synthetase	0.81	30
		Folate biosynthesis		
207.	MW0469	Dihydropteroate synthase	1.3	28
		Porphyrin and chlorophyll metabolism		
208.	MW1616	Glutamyl-tRNA reductase	0.21	28
209.	MW1613	Uroporphyrinogen III synthase	0.34	26
210.	MW2320	Uroporphyrin-III C-methyl transferase	1.2	26
211.	MW2539	Precorrin-2 dehydrogenase	1.7	28
		Terpenoid backbone biosynthesis		
212.	MW0450	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (EC:2.7.1.148)	5.1	35
213.	MW2466	Hydroxymethylglutaryl-CoA reductase	2.00E-005	21
214.	MW0545	Mevalonate kinase	3.00E-005	22
214.	MW0547	Phosphomevalonate kinase	7.3	26
		Nitrogen metabolism		
215.	MW1051	Carbamate kinase (EC:2.7.2.2)	0.12	39
216.	MW2553	Carbamate kinase (EC:2.7.2.2)	0.061	25
217.	MW2319	Respiratory nitrate reductase alpha chain	3.3	43
218.	MW2318	Nitrate reductase beta chain NarH	1.7	31
219.	MW2316	Nitrate reductase gamma chain	0.68	29
220.	MW0426	Glutamate synthase large subunit	0.5	24

Table 2: List of Essential Genes for CA-MRSA

Sr. No.	Gene ID	Gene Name	DEG ID	TARGET	PATHWAYS
1.	MW2049	**fbaA**	DEG10020239	**Fructose-bisphosphate aldolase** (EC:4.1.2.13)	Glycolysis / Gluconeogenesis Pentose phosphate pathway Fructose and mannose metabolism Methane metabolism
2.	MW0737	**pgm**	DEG10020010	**Phosphoglyceromutase** (EC:5.4.2.1)	Glycolysis / Gluconeogenesis Glycine, serine and threonine metabolism Methane metabolism
3.	MW2244	**glvC**	DEG10020290	PTS system arbutin-like IIBC component	Glycolysis / Gluconeogenesis
4.	MW1030	**sdhC**	DEG10150209	Succinate dehydrogenase cytochrome b-558	TCA cycle Oxidative phosphorylation Butanoate metabolism
5.	MW0844	**pgi**	DEG10020081	Glucose-6-phosphate isomerase (EC:5.3.1.9)	Pentose phosphate pathway Amino sugar and nucleo-tide sugar metabolism
6.	MW0113	**drm**	DEG10020010	Phosphopentomutase (EC:5.4.2.7)	Pentose phosphate pathway Purine metabolism
7.	MW2085	**mtlD**	DEG10020244	Mannitol-1-phosphate 5-dehydrogenase	Fructose and mannose metabolism
8.	MW0662	**fruA**	DEG10020060	Fructose specific permease	Fructose and mannose metabolism
9.	MW0223	**gatC**	DEG10170276	PTS galactitol-specific enzyme IIC component	Galactose metabolism
10.	MW0865	**fabH**	DEG10210185	3-oxoacyl-(acyl carrier protein) synthase III (EC:2.3.1.41)	Fatty acid biosynthesis
11.	MW0952	**purL**	DEG10100130	Phosphoribosylformylglycinamidine synthase II	Purine metabolism
12.	MW0110	**pnp**	DEG10020139	**Purine nucleoside phosphorylase** (EC:2.4.2.1)	Purine metabolism Pyrimidine metabolism Nicotinate and nicotinamide metabolism
13.	MW2062	**deoD**	DEG10060036	Purine nucleoside phosphorylase (EC:2.4.2.1)	Purine metabolism Pyrimidine metabolism Nicotinate and nicotinamide metabolism

14.	MW2143	**rpoA**	DEG10020252	DNA-directed RNA polymerase subunit alpha (EC:2.7.7.6)	Purine metabolism Pyrimidine metabolism
15.	MW1646	**dnaE**	DEG10020201	DNA polymerase III alpha subunit	Purine metabolism Pyrimidine metabolism
16.	MW1538	**holA**	DEG10170216	DNA polymerase III subunit delta	Purine metabolism Pyrimidine metabolism
17.	MW1141	**pyrH**	DEG10170157	**Uridylate kinase** (EC:2.7.4.22)	Pyrimidine metabolism
18.	MW0437	**tmk**	DEG10020028	Thymidylate kinase	Pyrimidine metabolism
19.	MW1259	**trpB**	DEG10020152	**Tryptophan synthase subunit beta** (EC:4.2.1.20)	Glycine, serine and threonine metabolism Phenylalanine, tyrosine and tryptophan biosynthesis
20.	MW2005	**murF**	DEG10020229	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopime-late-D-alanyl-D-alanyl ligase	Lysine biosynthesis Peptidoglycan biosynthesis
21.	MW0543	**eutD**	DEG10020053	Phosphotransacetylase	Taurine and hypotaurine metabolism Pyruvate metabolism Propanoate metabolism Methane metabolism
22.	MW1654	**ackA**	DEG10020202	**Acetate kinase** (EC:2.7.2.1)	Taurine and hypotaurine metabolism Pyruvate metabolism Propanoate metabolism Methane metabolism
23.	MW1033	**murI**	DEG10020105	Glutamate racemase	D-Glutamine and D-glutamate metabolism
24.	MW1066	**murD**	DEG10020108	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase (EC:6.3.2.9)	D-Glutamine and D-glutamate metabolism Peptidoglycan biosynthesis
25.	MW1683	**murC**	DEG10020208	UDP-N-acetylmuramate--L-alanine ligase (EC:6.3.2.8)	D-Glutamine and D-glutamate metabolism Peptidoglycan biosynthesis
26.	MW2006	**ddl**	DEG10020230	D-alanyl-alanine synthetase A (EC:6.3.2.4)	D-Alanine metabolism Peptidoglycan biosynthesis
27.	MW0454	**glmU**	DEG10170026	Bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	Amino sugar & nucleotide sugar metabolism
28.	MW2024	**murA**	DEG10020231	**UDP-N-acetylglucosamine 1-carboxyvinyltransferase** (EC:2.5.1.7)	Amino sugar & nucleotide sugar metabolism Peptidoglycan biosynthesis
29.	MW0700	**murB**	DEG10170067	UDP-N-acetylenolpyruvoylglucosamine reductase	Amino sugar & nucleotide sugar metabolism Peptidoglycan biosynthesis
30.	MW2459	**ptsG**	DEG10020290	PTS system glucose-specific IIABC component	Amino sugar & nucleotide sugar metabolism
31.	MW0645	**uppP**	DEG10020058	Undecaprenyl pyrophosphate phosphatase (EC:3.6.1.27)	Peptidoglycan biosynthesis
32.	MW1065	**mraY**	DEG10020107	Phospho-N-acetylmuramoyl-pentapeptide-transferase	Peptidoglycan biosynthesis
33.	MW1112	**plsX**	DEG10020118	Putative glycerol-3-phosphate acyltransferase PlsX	Glycerolipid metabolism Glycerophospholipid metabolism
34.	MW2590	**lip**	DEG10020080	Triacylglycerol lipase precursor (EC:3.1.1.3)	Glycerolipid metabolism
35.	MW0469	**folP**	DEG10070125	Dihydropteroate synthase	Folate biosynthesis
36.	MW2466	**mvaA**	DEG10210047	Hydroxymethylglutaryl-CoA reductase	Terpenoid backbone biosynthesis
37.	MW0545	**mvaK1**	DEG10210043	Mevalonate kinase	Terpenoid backbone biosynthesis
38.	MW0547	**mvaK2**	DEG10210045	Phosphomevalonate kinase	Terpenoid backbone biosynthesis
39.	MW2319	**narG**	DEG10020284	Respiratory nitrate reductase alpha chain	Nitrogen metabolism