Table S1. Predicted proteins associated with DAP resistance in *E. faecium* strains

E. faecium DO			Presumptive annotation /Comments	Species	Ref.
Locus	**Locus Tag**	**Accesion Number**			
LiaF	HMPREF0351_10936	YP_006375542	Cell wall-active antibiotics response protein_LiaF/YvqF A group of cell wall-active antibiotic response proteins, including LiaF and YvqF types.	*S. mutants* *S.pneumoniae* *B. subtilis* *S. aureus*	(1)
LiaS	HMPREF0351_10937	YP_006375543	Signal transduction histidine kinase, LiaS Two-component sensor responding to cell wall stress.	*S. mutants* *S.pneumoniae* *B. subtilis* *S. aureus*	(1)
LiaR	HMPREF0351_10938	YP_006375544	Response regulator domain VraR/LiaR Two-component response regulator [YvqE] responding to cell wall stress.	*S. mutants* *S.pneumoniae* *B. subtilis* *S. aureus*	(1)
Cls	HMPREF0351_11068	YP_006375674	Putative cardiolipin synthetase Cardiolipin synthases that belong to the phospholipase D family. These enzymes catalyze the reversible tgransfer of one phosphatidyl group from one phosphatidylglycerol molecule to another to form cardiolipin (diphosphatidylglycerol) and glycerol.	*E.faecium.* *E.faecalis* *E. hirae* *E. mundtii* *E. durans* *E. villorum*	(1)
GdpD	HMPREF0351_11730	YP_006376336	Glycerophosphodiester phosphodiesterase family protein This subfamily corresponds to the glycerophosphodiester phosphodiesterase domain (GDPD) present in uncharacterized bacterial glycerophosphodiester phosphodiesterases.	*E.faecium.* *E.faecalis* *E. villorum* *E. hirae* *E. mundtii* *E. durans*	(1)
Cfa	HMPREF0351_11433	YP_006376039	Cyclopropane-fatty-acyl-phospholipid synthase Fatty-acyl-phospholipid/mycolic acid cyclopropane synthase	*E .faecium.* *E. durans*	(2)
PTS-EIIA	HMPREF0351_11023	YP_006375629	Phosphotransferase system, phosphoenolpyruvate-dependent sugar EIIA 2		(2)
YycF/WalR/VicR	HMPREF0351_12362	YP_006376968	Response regulator transcriptional regulatory protein WalR/yycF	*E. faecium.* *E. faecalis* *E. villorum* *E. hirae* *E. mundtii* *E. durans*	(2)
YycG/Walk/VicK	HMPREF0351_12361	YP_006376967	Signal transduction histidine		(2)

			kinase domain protein yycFG Vick/walK		
YycH	HMPREF0351_12360	YP_006376966	YycH protein YycH plays a role in signal transduction and is found immediately downstream of the essential histidine kinase YycG.		(2)
YycI	HMPREF0351_12359	YP_006376965	YycI protein Regulatory protein		(2)
YycJ	HMPREF0351_12358	YP_006376964	Metallo-beta-lactamase YycJ Metal-dependent hydrolases of the beta-lactamase superfamily I		(2)
HD Domain protein	HMPREF0351_11908	YP_006376514	HD domain protein The HD domain is found in a superfamily of enzymes with a predicted or known phosphohydrolase activity. These enzymes appear to be involved in nucleic acid metabolism, signal transduction and possibly other functions in bacteria, archaea and eukaryotes. The fact that all the highly conserved residues in the HD superfamily are histidines or aspartates suggests that coordination of divalent cations is essential for the activity of these proteins	*E. faecium*	(2)
SulP	HMPREF0351_10763	YP_006375369	Sulphate transporter A number of proteins involved in the transport of sulphate across the cell membrane	*E. faecium*	(2)
XpaC	HMPREF0351_10542	YP_006375148	5-bromo-4-chloroindolyl phosphate hydrolysis protein Members of this family of prokaryotic proteins mediate the hydrolysis of 5-bromo-4-chloroindolyl phosphate bonds	*E. faecium*	(2)
RrmA	HMPREF0351_12412	YP_006377018	rRNA (guanine-N1-)-methyltransferase A, predicted protein These enzymes specifically methylate the guanosine residue m1G in 23S rRNA.	*E. faecium*	(2)
BetaFructoronidase (Glycoside hydrolase, family 32)	HMPREF0351_11791	YP_006376397	Glycoside hydrolase, family 32 (GH32). GH32 enzymes cleave sucrose into fructose and glucose via beta-fructofuranosidase activity, producing an invert sugar that is a mixture of dextrorotatory D-glucose and levorotatory D-	*E. faecium* *E. avium*	(3)

			fructose, thus named invertase		
PTS-IIA	HMPREF0351_11792	YP_006376398	PTS system fructose IIA component	*E. faecium* *E. avium*	(3)
Bacteriophage Minor	HMPREF0351_10870	YP_006375476	Phage minor structural protein. A conserved domain found in a number of uncharacterized phage proteins.	*E. faecium*	(3)
PspC	HMPREF0351_12014	YP_006376620	PspC domain Phage shock protein C (PspC) that is thought to be a transcriptional regulator.	*E. faecium*	(3)
LepB	HMPREF0351_11130	YP_006375736	Signal peptidase I This group of serine peptidases belong to MEROPS (peptidase database) peptidase family S26 (signal peptidase I family, clan SF), subfamily S26A.	*E. faecium*	(3)
Hypothetical Protein	HMPREF0351_11658	YP_006376264	Putative Lipoprotein Prokaryotic membrane lipoprotein lipid attachment site.	*E. faecium*	(3)
Aad	HMPREF0351_10204	YP_006374810	Aldehyde-alcohol dehydrogenase Bifunctional aldehyde-alcohol dehydrogenase	*E. faecium* *E .hirae* *E. mundtii* *E. durans*	(3)
NrdR	HMPREF0351_10668	YP_006375274	Nicotinamide mononucleotide transporter PnuC PnuC is a membrane protein responsible for nicotinamide mononucleotide transport Members of this family are integral membrane proteins that are involved in transport of nicotinamide mononucleotide	*E. faecium* *E. durans*	(3)
PTS IID	HMPREF0351_12883	YP_006377489	PTS system mannose/fructose/sorbose transporter subunit IID	*E. faecium*	(3)
EzrA	HMPREF0351_12190	YP_006376796	Septation ring formation regulator EzrA During the bacterial cell cycle, the tubulin-like cell-division protein FtsZ polymerizes into a ring structure that establishes the location of the nascent division site. EzrA modulates the frequency and position of FtsZ ring formation.	*E .faecium.* *E. durans* *E. hirae*	(3)
AlfaMannosidase (Glycoside hydrolase family 38)	HMPREF0351_12498	YP_006377104	Glycoside hydrolase family 38 GH38. Comprises enzymes with only one known activity, alpha-mannosidase	*E. faecium*	(3)

Outer Surface Protein	HMPREF0351_11298	YP_006375904	This predicted protein harbors two domains; ***i***) cyclophilin-like peptidyl-prolyl cis-trans isomerase domain Aldolase-type and ***ii***) a TIM barrel domain. Cyclophilins exhibit peptidyl-prolyl cis-trans isomerase (PPIase) activity. TIM barrel usually covers the entire protein structure	*E. faecium* *E. faecalis* *E .hirae* *E. durans*	(3)
Hypothetical Protein	HMPREF0351_10875	YP_006375481	Conserved hypothetical protein No significant domains	*E. faecium* *E. mundtii*	(3)
Hypothetical protein	HMPREF0351_10830	YP_006375436	Conserved hypothetical protein	*E. faecium*	(3)
RpeS – 5S	HMPREF0351_10075	YP_006374681	Ribosomal protein 5S	*E. faecalis* *E. faecium* *E.saccharolyticus* *E. casseliflavus* *E. villorum* *E. caccae*	(4)
BrnQ	HMPREF0351_10149	YP_006374755	LIVCS family branched chain amino acid:cation symporter Transport. All three of the branched chain aliphatic amino acids (leucine (L), isoleucine (I) and valine (V)). They function by a Na+ or H+ symport mechanism and display 12 putative transmembrane helical spanners.	*E. faecalis* *E. faecium*	(4)
RpoN	HMPREF0351_12069	YP_006376675	RNA polymerase sigma factor 54 (IPR000394)	*E. faecalis* *E. faecium*	(4)
Hypothetical Protein EF1753	HMPREF0351_12015	YP_006376621	Conserved hypothetical protein No transmembrane domains, No signal peptide.	*E. faecium*	(4)
Hypothetical Protein EF1797	HMPREF0351_10297	YP_006374903	Conserved hypothetical protein 5 - 6 transmembrane domains and signal-peptide	*E. faecium*	(4)
Hypothetical Protein EF1797 -2	HMPREF0351_10318	YP_006374924	Conserved hypothetical protein 5-6 transmembrane domains and signal-peptide	*E. faecium*	(4)
TelA	HMPREF0351_10540	YP_006375146	This family consists of several prokaryotic TelA-like proteins. TelA and KlA are associated with tellurite resistance and plasmid fertility inhibition	*E. faecium* *E.faecalis* *E .hirae* *E. mundtii* *E. durans* *E. villorum*	(4)
MprF/FmtC	HMPREF0351_11082	YP_006375688	Lysylphosphatidylglycerol synthetase. Proteins involved in the addition of lysine to phosphatidylglycerol (lysylphosphatidylglycerol synthetase.	*E. faecium* *S. aureus*	(5, 6)

PgsA	HMPREF0351_12591	YP_006377197	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase Catalyses the conversion of CDP-diacylglycerol and glycerol-3-phosphate to CMP and 3-(3-phosphatidyl)-glycerol 1-phosphate in the committed step to the synthesis of acidic phospholipids. It is an integral membrane protein.	*E. faecium* *E .hirae* *E. mundtii* *E. durans* *S. aureus*	(7)
DltA	HMPREF0351_10751	YP_006375357	D-alanyl carrier protein ligase: DltA A domain responsible for the specific recognition of amino acids and activation as adenylyl amino acids.	*S. aureus* *E. faecium*	(6)
DltB	HMPREF0351_10752	YP_006375358	DltB is an alginate O-acetyltransferase AlgI/D-alanyl transfer D-alanyl transfer protein It is predicted to be a transmembrane protein and is thought to be involved in the D-alanine incorporation into teichoic acids and in the transfer of activated D-alanine across the cytoplasmic membrane.	*E. faecium* *S. aureus*	(6)
DltC	HMPREF0351_10753	YP_006375359	D-Alalnyl carrier protein DltC.	*E. faecium* *S. aureus*	(6)
DltD	HMPREF0351_10754	YP_006375360	D-alanyl-lipoteichoic acid biosynthesis DltD: involved in the transfer of D-alanine from the membrane carrier to teichoic acids.	*E. faecium* *S. aureus*	(6)

Table S2. *E. faecium* homologues of predicted proteins associated with DAP resistance in *E. faecalis* and *S. aureus*

Predicted protein	Organism	Closest homolog in *E. faecium* DO	Amino acid identity (%)	Amino acid homology (%)	E value
EF0224, RpsE	*E. faecalis* V583	HMPREF0351_10075	98	98	4e-111
EF0243, BrnQ	*E. faecalis* V583	HMPREF0351_10149	67	81	00
EF0782, RpoN	*E. faecalis* V583	HMPREF0351_12069	63	91	00
EF1753	*E. faecalis* V583	HMPREF0351_12015	59	74	00
EF1797	*E. faecalis* V583	HMPREF0351_10297	46	65	1e-50
		HMPREF0351_10318	43	64	1e-47
EF2698, TelA	*E. faecalis* V583	HMPREF0351_10540	83	91	00
MprF/FmtC	*S. aureus* COL	HMPREF0351_11082	30	51	4e-105
PgsA	*S. aureus* COL	HMPREF0351_12591	48	65	4e-52
DltA	*S. aureus* COL	HMPREF0351_10751	41	59	7e-120
DltB	*S. aureus* COL	HMPREF0351_10752	47	63	2e-105
DltC	*S. aureus* COL	HMPREF0351_10753	42	77	5e-19
DltD	*S. aureus* COL	HMPREF0351_10754	29	50	1e-47

Table S3. More frequent amino acid (AA) substitutions in *E. faecium* strains

Predicted protein	AA Change	# (%) of strains (Total=19)	DAP MIC/Range of MICs (µg/ml)	Strains
LiaS	Thr120→Ala	7	3 – 48	503, 506, 513, R494, R497, V689, P1190
	Leu70→Phe	1	4	504
	Asp251→Asn	1	6	505
	Total	**9(47%)**	**3 - 48**	
LiaR	Trp73→Cys	7	3-48	503, 506, 513, R494, R497, V689, P1190
	Total	**7(37%)**	**3 - 48**	
Cls	Arg218→Gln	2	16-32	R446, R501
	His215→Arg	1	48	R499
	Asn13→Ile	1	32	R496
	Insertion110 of Met-Pro-Leu	1	16	R497
	Asn237→Asp/Glu279→Gln	1	6	505
	Gly181→Ser /Ser455→Leu	1	4	504
	Total	**7(37%)**	**4-48**	
HD domain protein	Ala151→Pro	3	4 – 48	515, V689, R494
	Arg38→His	1	16	R446
	His35→Pro/deletion of Ser36	1	48	R501
	Total	**5(26%)**	**4-48**	
LiaF	Asn110→Ser	1	6	505
	Ile142→Thr	1	32	R496
	Leu39→Phe	1	48	R494
	Thr35→Ile	1	4	504
	Total	**4(21%)**	**4-48**	
RrmA	Ala576→Gly	1	6	505
	Glu204→Val	1	4	504
	Ser76→Tyr	1	16	R44
	Total	**3(16%)**	**4-16**	
YycH	Asn429→Ser	1	6	506
	Asp311→Glu	1	48	R501
	Gly386→Arg	1	4	504
	Total	**3(16%)**	**4-48**	
Hypothetical Protein	Ala12→Thr / Leu14→Phe	1	6	506
	Del55→Thr / Glu56→Val	1	3	P1190
	Total	**3(16%)**	**3-6**	
RpoN	Arg224→Gln	1	4	504
	Del AA1-87	1	48	R501
	Pro137→Ser/Glu322→Lys/Asn444→His	1	6	505
	Total	**3(16%)**	**4-48**	

TelA	Asp20→Tyr	1	6	506
	Leu299→Phe	2	4	513
	Total	**3(16%)**	**4-6**	
Cfa	Gly292→Ala	1	2	S447
	Tyr7→Cys	1	4	509
	Total	**2(11%)**	**2-4**	
PTS- EIIA	Lys111→Asn	1	2	S447
	Pro14→Ser /Asp34→Asn	1	6	505
	Total	**2(11%)**	**2-6**	
YycG/Walk/Vic K	Ala414→Thr	1	3	515
	Ser333→Leu	1	16	R446
	Total	**2(11%)**	**3-16**	
YycI	Asn185→Ser	1	6	505
	Gly228→Glu	1	24	R499
	Total	**2(11%)**	**6-24**	
Bacteriophage minor structural protein	>17 changes	1	3	P1190
	5 changes	1	4	510
	Total	**2(11%)**	**3-4**	
Aad (aldehyde alcohol dehydrogenase)	Asp161→Ala	1	4	504
	Val121→Gly	1	6	506
	Total	**2(11%)**	**4-6**	
Alfa Mannosidase (Glycoside hydrolase family 38)	Gln767→His	1	4	504
	>9changes	1	6	505
	Total	**2(11%)**	**4-6**	
Hypothetical Protein	Arg96→Ser	1	4	504
	Thr29→Ala/His44→Arg/Ser55→Arg/Met62→Thr/Leu61→Ile	1	6	505
	Total	**2(11%)**	**4-6**	
MprF	Thr416→Pro	1	4	504
	Lys744→Glu /Ala760→Ser	1	6	505
	Total	**2 (11%)**	**4 - 6**	
GdpD	Ile283→Phe	1	48	R494
	Total	**1 (5%)**	**48**	
SulP	Hys151→Tyr	1	16	R446
	Total	**1 (5%)**	**16**	
XpaC	Asp198→His	1	2	S447
	Total	**1 (5%)**	**2**	
PspC	Lys3→Glu	1	64	R494
	Total	**1 (5%)**	**64**	
LepB	Truncated	1	32	R496
	Total	**1 (5%)**	32	
NrdR	Phe122→Leu	1	4	510
	Total	**1 (5%)**	**4**	
PTS IID	Ile43→Met	1	6	505
	Total	**1 (5%)**	**6**	
EzrA	Ile9→Met/Thr326→Ala	1	6	505
	Total	**1 (5%)**	**6**	
BrnQ	Val134→Gly /Leu409→Ile	1	6	505
	Total	**1 (5%)**	**6**	
Hypothetical	Ala290→Ser	1	4	504

protein	**Total**	**1 (5%)**	**4**	
DltA	Glu177→Lys	1	6	505
	Total	**1 (5%)**	**6**	
DltD	Ser410→Asp	1	6	505
	Total	**1 (5%)**	**6**	

References

1. **Arias, C. A., D. Panesso, D. M. McGrath, X. Qin, M. F. Mojica, C. Miller, L. Diaz, T. T. Tran, S. Rincon, E. M. Barbu, J. Reyes, J. H. Roh, E. Lobos, E. Sodergren, R. Pasqualini, W. Arap, J. P. Quinn, Y. Shamoo, B. E. Murray, and G. M. Weinstock.** 2011. Genetic basis for *in vivo* daptomycin resistance in enterococci. The New England journal of medicine **365:**892-900.
2. **Tran, T. T., D. Panesso, H. Gao, J. H. Roh, J. M. Munita, J. Reyes, L. Diaz, E. A. Lobos, Y. Shamoo, N. N. Mishra, A. S. Bayer, B. E. Murray, G. M. Weinstock, and C. A. Arias.** 2013. Whole-genome analysis of a daptomycin-susceptible *Enterococcus faecium* strain and its daptomycin-resistant variant arising during therapy. Antimicrob Agents Chemother. **57:**261-268.
3. **Humphries, R. M., T. Kelesidis, R. Tewhey, W. E. Rose, N. Schork, V. Nizet, and G. Sakoulas.** 2012. Genotypic and phenotypic evaluation of the evolution of high-level daptomycin nonsusceptibility in vancomycin-resistant *Enterococcus faecium*. Antimicrob Agents Chemother. **56:**6051-6053.
4. **Palmer, K. L., A. Daniel, C. Hardy, J. Silverman, and M. S. Gilmore.** 2011. Genetic basis for daptomycin resistance in enterococci. Antimicrob Agents Chemother. **55:**2245-2256.
5. **Bao, Y., T. Sakinc, D. Laverde, D. Wobser, A. Benachour, C. Theilacker, A. Hartke, and J. Huebner.** 2012. Role of *mprF1* and *mprF2* in the pathogenicity of *Enterococcus faecalis.* Plos One **7:**e38458.
6. **Bayer, A. S., T. Schneider, and H. G. Sahl.** 2013. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. Ann N Y Acad Sci **1277:**139-158.
7. **Peleg, A. Y., S. Miyakis, D. V. Ward, A. M. Earl, A. Rubio, D. R. Cameron, S. Pillai, R. C. J. Moellering, and G. M. Eliopoulos.** 2012. Whole genome characterization of the mechanisms of daptomycin resistance in clinical and laboratory derived isolates of *Staphylococcus aureus.* Plos One **7:**e28316.