

Key for Web Exploration Questions, Chapter 9

Report your findings regarding antibiotic resistance in the *Enterococcus faecium* strain isolated from the abdominal infection. Discuss whether this strain is multi-drug resistant and what antibiotics it is resistant to. Then, provide an annotated list of genes on the plasmid for which you have solid evidence: name them if possible (refer to them by the starting position of the ORF where you can't find a suitable name), give their length in amino acids and list their functions briefly but specifically.

The annotated gene list below is derived from the list of ORFs found by NEBcutter, which does not list overlapping ORFs. Some additional short ORFs were found by NCBI's ORF Finder that were not investigated further. From the NEBcutter list, short ORFs that were not supported as potential expressed genes by EasyGene *and* showed no significant similarity in a BLAST search were deleted as very unlikely to be genes. The remaining potential genes include some unlikely possibilities but some that may have been mutated over time or by a transposition event.

Given the genes for which there is good evidence, it is clear that this plasmid would confer resistance to vancomycin (a very important "last resort" antibiotic), streptomycin and/or other aminoglycoside antibiotics, streptothricin and/or other antibiotics of its class, erythromycin and probably kanamycin and perhaps other antibiotics as well. Additionally, it is clear from the many genes with similarity to transposases that this plasmid was likely the result of multiple transposition events bringing together the various resistance genes.

Gene	Strand	Start	End	Length		Function and Notes
				nt	aa	
<i>pinR</i>	as entered	38	532	495	164	Resolvase, in serine resolvase family; catalyzes recombination between the two ends of a transposon, required for replicative transposition ("copy" mechanism of transposon "hop")
<i>vanR</i>	as entered	746	1441	696	231	Response regulator: part of a two-component regulatory system (along with VanS) that induces vancomycin resistance genes in response to vancomycin. Not supported by EasyGene, but supported by strong BLAST similarity.
<i>vanS</i>	as entered	1478	2572	1095	364	Sensor kinase: part of a two-component regulatory system (along with VanR) that induces vancomycin resistance genes in response to vancomycin. Not supported by EasyGene, but supported by strong BLAST similarity.
<i>vanH</i>	as entered	3225	3755	531	176	Vancomycin resistance: catalyzes conversion of pyruvate to D-lactate, which can be used by VanA to synthesize vancomycin-resistant peptidoglycan peptides. EasyGene finds ATG start at 3225; start at 2787 (322 aa) found by NEBcutter and ORF Finder but not supported by EasyGene.
<i>vanA</i>	as entered	3748	4779	1032	343	Vancomycin resistance: catalyzes addition of D-lactate to D-alanine to make vancomycin-resistant peptidoglycan peptides.
<i>vanX</i>	as entered	4785	5393	609	202	Vancomycin resistance, working along with VanH and Van A: cleaves D-alanine-D-alanine peptides to remove non-resistant peptidoglycan peptides, leaving resistant D-alanine-D-lactate peptides.

Gene	Strand	Start	End	Length		Function and Notes
				nt	aa	
<i>vanY?</i>	as entered	5736	5876	141	46	Very strong similarity in a very short region to VanY carboxypeptidases which work along with VanA, VanH and VanX to remove vancomycin-susceptible peptides from peptidoglycan. Far too short to be an actual VanY gene; maybe the result of a frameshift or other mutational event? Not supported by EasyGene as a gene.
<i>vanY</i>	as entered	5821	6366	546	181	VanY carboxypeptidase: works along with VanA, VanH and VanX to remove vancomycin-susceptible peptides from peptidoglycan.
<i>ermAM</i>	as entered	7963	8700	738	245	Erythromycin resistance: methyltransferase which methylates the ribosomal RNA target of erythromycin so that erythromycin cannot bind and inhibit translation
	as entered	8846	9130	285	94	Very strong similarity to DNA topoisomerases, but only in a limited region. Perhaps a fragment of a gene due to a frameshift or a transposition event occurring in the middle of a gene? Not supported as a gene by EasyGene.
<i>Tnp167A</i>	as entered	9647	10183	537	178	Transposase: functions in transposition ("hop") of transposons with IS3 ends. EasyGene finds TTG start at 9647 rather than ATG start at 9857 (109aa) found by ORF Finder; not found by NEBcutter due to overlap.
<i>InsK</i>	as entered	10180	10986	807	268	Transposase: functions in transposition ("hop") of transposons with IS3 ends. EasyGene finds TTG start at 10180 rather than ATG at 10084 found by ORF Finder and NEBcutter giving (300 aa).
transposase	as entered	12234	12908	675	224	Transposase: functions in transposition ("hop") of transposons with IS4 ends.
	as entered	12935	13180	246	81	Similar to predicted but not yet identified proteins containing ATP-binding domains associated with other antibiotic-resistance plasmids. Function not yet known. Not supported as a gene by EasyGene, so uncertain if expressed.
KNTase	as entered	13283	14152	870	289	Probably kanamycin resistance: contains a nucleotidyltransferase domain similar to resistance proteins of other bacteria which transfer a nucleotide to the antibiotic kanamycin, inactivating it.
	as entered	14133	14867	735	244	Strong similarity to methyltransferases, but the substrate of the methyltransferase cannot be determined from the similarity results. Could potentially methylate some antibiotic and inactivate it (like ermAM).
	as entered	14842	14955	114	37	Strong similarity to a conserved by hypothetical protein found in <i>Staphylococcus</i> . Not supported by EasyGene, can't determine if expressed or if a full-length gene.
<i>aadE</i>	as entered	14900	15808	909	302	Adenyltransferase: adanylates and inactivates streptomycin and other aminoglycoside antibiotics. Not supported by EasyGene even though clearly a long ORF with good similarity.
<i>rimI</i>	as entered	15817	16347	531	176	Acetyltransferase that acetylates streptothricin and other antibiotics of its class, inactivating them. Not supported by EasyGene even though clearly a long ORF with good similarity.

Gene	Strand	Start	End	Length		Function and Notes
				nt	aa	
<i>aph</i>	as entered	16440	17234	795	264	Aminoglycoside phosphotransferase: phosphorylates and inactivates aminoglycoside antibiotics such as gentamycin and streptomycin.
	as entered	17268	17483	216	71	Modest similarity in the downstream half of the ORF to proteins of the LysM family; this domain has been found in proteins that degrade bacterial cell walls and as signals for bacteria-plant recognition. Its function in this plasmid is unclear; the ORF is not supported as a gene by EasyGene, so it could be a fragment or unexpressed.
	complement	5477	5641	165	54	Modest similarity to a hypothetical protein found in other <i>Enterococcus</i> strains but with no known function. Not supported as a gene by EasyGene, could be a gene fragment or unexpressed mutant.
	complement	5651	5779	129	42	Weak similarity in a very short region to a chitin-binding protein. Not supported by EasyGene.
	complement	6297	6410	114	37	Strong similarity in a very short region to a ribonucleoside-diphosphate reductase. Not supported by EasyGene
transposase	complement	6389	7069	681	226	Transposase: functions in transposition ("hop") of transposons with IS431 ends. EasyGene finds ATG start at 7069; start at 7075 (687aa) found by NEBcutter and ORF Finder not supported.
	complement	7404	7862	459	152	Weak similarity to hypothetical proteins identified in other genomes. Not supported by EasyGene despite being a long ORF, perhaps not a gene.
	complement	8680	8904	225	74	Weak similarity to hypothetical proteins and a ribosomal protein. Not supported by EasyGene.
	complement	9111	9305	195	64	Fair similarity to a glycosyltransferase (which would add sugars to proteins or build polysaccharides) and to hypothetical proteins. Not supported by EasyGene.
transposase	complement	9287	9430	144	47	Moderate similarity to a transposase that works on transposons with IS1167 ends, not supported by EasyGene.
	complement	9820	10110	291	96	Weak similarity to cytochrome b. Not supported by EasyGene.
transposase	complement	10988	12001	1014	337	Transposase: functions in transposition ("hop") of transposonse with IS3L ends.
	complement	12147	12278	132	43	Moderate similarity to a hypothetical protein of <i>Staphylococcus</i> . Not supported by EasyGene.
	complement	12864	12956	93	30	Weak similarity to transporters and cell-wall anchor proteins. Not supported by EasyGene.