

**Table S1. Bacterial strains used in this study.**

	<b>Strain identifier</b>	<b>Strain common name used in main text (chromosomal antibiotic resistance<sup>a</sup>)</b>	<b>Relevant phenotypic/genotypic strain characteristics<sup>b</sup></b>	<b>Plasmid(s) (section of Table S2)<sup>d</sup></b>	<b>Reference / source</b>
<b>A. E. faecalis laboratory strains</b>	SJH-1994	OG1RF (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup>	None	(1)
	SJH-1220	OG1X (Str)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup>	None	(2)
<b>B. E. faecalis OG1RF markerless, in-frame chromosomal mutants</b>	SJH-953	SrtA <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>-</sup> ( $\Delta$ srtA <sup>c</sup> )	None	(3)
	SJH-1421	EbpC <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpC)	None	This study
	SJH-1422	EbpABC <sup>-</sup> (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>-</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABC)	None	This study
	SJH-1424	SrtC <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>-</sup> , SrtA <sup>+</sup> ( $\Delta$ srtC)	None	This study
	SJH-1995	EbpABC <sup>-</sup> SrtC <sup>-</sup> (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>-</sup> , EbpC <sup>-</sup> , SrtC <sup>-</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABCsrtC)	None	This study
	SJH-1996	EbpA <sup>-</sup> (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpA)	None	This study
	SJH-1997	EbpB <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>-</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpB)	None	This study
	SJH-1998	EbpBC <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>-</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpBC)	None	This study
	SJH-1999	EbpAC <sup>-</sup> (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>+</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpA, $\Delta$ ebpC)	None	This study
	SJH-2000	EbpAB <sup>-</sup> (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>-</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpAB)	None	This study
	SJH-2001	EbpA <sup>AWAGA</sup> (Rif/Fus)	EbpA <sup>D315A, S317A, S319A</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> (ebpA <sup>AWAGA</sup> )	None	This study
	SJH-2010	SrtC <sup>-</sup> SrtA <sup>-</sup> (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>-</sup> , SrtA <sup>-</sup> ( $\Delta$ srtC, $\Delta$ srtA <sup>c</sup> )	None	This study
<b>C. E. faecalis mutant strains with the empty vector</b>	SJH-2020	EbpABC <sup>-</sup> /pGCP123 (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>-</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABC)	pSJH-329 (D)	This study
	SJH-2187	EbpABC <sup>-</sup> SrtC <sup>-</sup> /pGCP123 (Rif/Fus)	EbpA <sup>-</sup> , EbpB <sup>-</sup> , EbpC <sup>-</sup> , SrtC <sup>-</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABCsrtC)	pSJH-329 (D)	This study
<b>D. E. faecalis mutant strains expressing ebp genes in trans</b>	SJH-2011	EbpABC <sup>-</sup> / p-ebpABC (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABC)	pSJH-491 (I)	This study
	SJH-2014	EbpABC <sup>-</sup> /p-ebpAB (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>-</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABC)	pSJH-492 (I)	This study
	SJH-2030	EbpABC <sup>-</sup> SrtC <sup>-</sup> /p-ebpABCsrtC (Rif/Fus)	EbpA <sup>+</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABCsrtC)	pSJH-496 (I)	This study
	SJH-2220	EbpABC <sup>-</sup> SrtC <sup>-</sup> /p-ebpA <sup>AWAGA</sup> BCsrtC (Rif/Fus)	EbpA <sup>D315A, S317A, S319A</sup> , EbpB <sup>+</sup> , EbpC <sup>+</sup> , SrtC <sup>+</sup> , SrtA <sup>+</sup> ( $\Delta$ ebpABCsrtC)	pSJH-559 (I)	This study

	Strain identifier	Strain common name used in main text (chromosomal antibiotic resistance <sup>a</sup> )	Relevant phenotypic/genotypic strain characteristics <sup>b</sup>	Plasmid(s) (section of Table S2) <sup>c</sup>	Reference / source
<b>E. Commercial <i>E. coli</i> cloning strains</b>	SJH-933	TOP10	F <sup>'</sup> { <i>lacIq</i> , Tn10(Tet <sup>R</sup> )} <i>mcrA</i> Δ( <i>mrr-hsdRMS-mcrBC</i> ) Φ80 <i>lacZ</i> ΔM15 Δ <i>lacX74 recA1 araD139</i> Δ( <i>ara leu</i> ) 7697 <i>galU galK rpsL</i> (Str <sup>R</sup> ) <i>endA1 nupG</i>	None	Invitrogen
	SJH-1887	NovaBlue	<i>endA1 hsdR17</i> (r <sub>K12</sub> <sup>-</sup> m <sub>K12</sub> <sup>+</sup> ) <i>supE44 thi-1 recA1 gyrA96 relA1 lac</i> F'[ <i>proA<sup>+</sup>B<sup>+</sup> lacI<sup>f</sup>ZΔM15::Tn10</i> ] (Tet <sup>R</sup> )	None	Novagen
	SJH-1888	XL1-Blue	<i>endA1 gyrA96(nalR) thi-1 recA1 relA1 lac glnV44</i> F[ <i>::Tn10 proAB<sup>+</sup> lacI<sup>f</sup> Δ(lacZ)M15</i> ] <i>hsdR17</i> (r <sub>K</sub> <sup>-</sup> m <sub>K</sub> <sup>+</sup> )	None	Stratagene
<b>F. Commercial <i>E. coli</i> expression strains</b>	SJH-1889	M15/pREP4	NaI <sup>S</sup> , Str <sup>S</sup> , Rif <sup>S</sup> , Thi <sup>-</sup> , Lac <sup>-</sup> , Ara <sup>+</sup> , Gal <sup>+</sup> , Mtl <sup>-</sup> , F <sup>-</sup> , RecA <sup>+</sup> , Uvr <sup>+</sup> , Lon <sup>+</sup>	pREP4 (A)	Qiagen
	SJH-1890	SG13009/pREP4	NaI <sup>S</sup> , Str <sup>S</sup> , Rif <sup>S</sup> , Thi <sup>-</sup> , Lac <sup>-</sup> , Ara <sup>+</sup> , Gal <sup>+</sup> , Mtl <sup>-</sup> , F <sup>-</sup> , RecA <sup>+</sup> , Uvr <sup>+</sup> , Lon <sup>+</sup>	pREP4 (A)	Qiagen
<b>G. <i>E. coli</i> strains expressing recombinant Ebp pilins</b>	SJH-1987	M15/pQE-30Xa-EbpA-X/pREP4	NaI <sup>S</sup> , Str <sup>S</sup> , Rif <sup>S</sup> , Thi <sup>-</sup> , Lac <sup>-</sup> , Ara <sup>+</sup> , Gal <sup>+</sup> , Mtl <sup>-</sup> , F <sup>-</sup> , RecA <sup>+</sup> , Uvr <sup>+</sup> , Lon <sup>+</sup>	pREP4(A), pSJH-541 (J)	This study
	SJH-1988	M15/pQE-30Xa-EbpB-X/pREP4	NaI <sup>S</sup> , Str <sup>S</sup> , Rif <sup>S</sup> , Thi <sup>-</sup> , Lac <sup>-</sup> , Ara <sup>+</sup> , Gal <sup>+</sup> , Mtl <sup>-</sup> , F <sup>-</sup> , RecA <sup>+</sup> , Uvr <sup>+</sup> , Lon <sup>+</sup>	pREP4 (A), pSJH-547 (J)	This study
	SJH-1985	SG13009/pQE-30Xa-EbpCA-X/pREP4	NaI <sup>S</sup> , Str <sup>S</sup> , Rif <sup>S</sup> , Thi <sup>-</sup> , Lac <sup>-</sup> , Ara <sup>+</sup> , Gal <sup>+</sup> , Mtl <sup>-</sup> , F <sup>-</sup> , RecA <sup>+</sup> , Uvr <sup>+</sup> , Lon <sup>+</sup>	pREP4 (A), pSJH-550 (J)	This study

<sup>a</sup> Rif (100 ug mL<sup>-1</sup>), Fus (fusidic acid, 100 ug mL<sup>-1</sup>), Str - streptomycin (500 μg mL<sup>-1</sup>)

<sup>b</sup> For *E. faecalis* strains generated in this study (sections B-D), any mutant allele present in the genome is listed in parentheses and described in Table S4 (sections A-B).

<sup>c</sup> The Δ*srtA* allele is described in (4).

<sup>d</sup> A letter in parentheses refers to the section of Table S2 where the noted plasmid is described.

## REFERENCES

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