

Supplemental materials

The two component system ChtRS contributes to chlorhexidine tolerance in *Enterococcus faecium*

Ana M. Guzmán Prieto, Jessica Wijngaarden, Johanna C. Braat, Malbert R.C. Rogers, Eline Majoor, Ellen C. Brouwer, Xinglin Zhang^{*}, Jumamurat R. Bayjanov, Marc J.M. Bonten, Rob J.L. Willems, Willem van Schaik^{a#}.

Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands.

Running title: ChtRS regulates chlorhexidine tolerance in *E. faecium*

Address correspondence to Willem van Schaik, w.vanschaik@umcutrecht.nl.

*Present address: College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China.

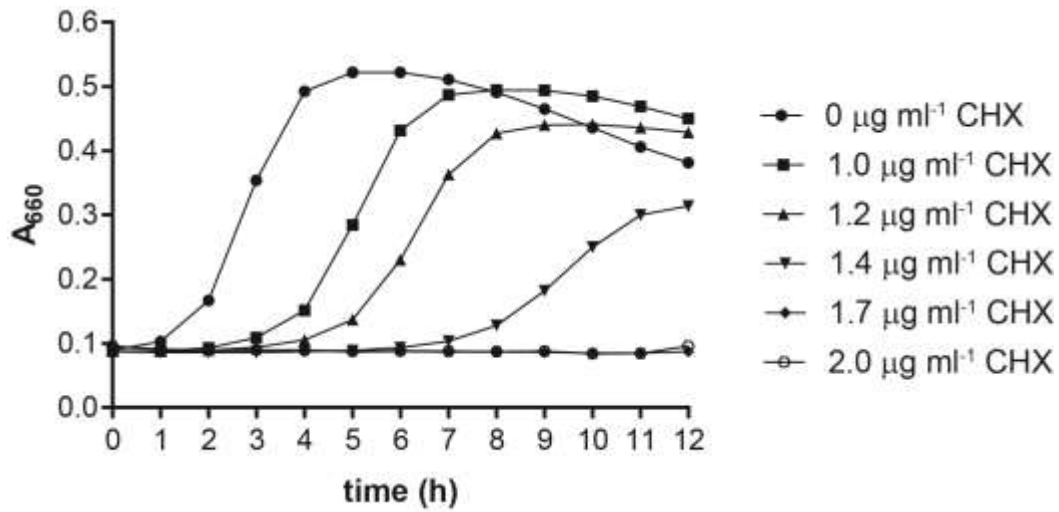


Fig S1. Growth of *E. faecium* E1162 in different concentrations of CHX. Growth curves of *E. faecium* E1162 challenged with different concentrations of CHX. The growth curves represent the averages of three replicates.

Table S1: Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)		Reference
<i>E. faecium</i>		Clade	
E1162	Clinical isolate (blood infection); Amp ^r , Tet ^r	A-1	61
E980	Isolated from feces of a non-hospitalized individual	B	61
E1590	Isolated from feces of a non-hospitalized individual	B	3
E7345	Clinical isolate (bloodstream infection) Aus0004; Van ^r	A-1	60
E745	Clinical isolate (bloodstream infection) ; Van ^r	A-1	unpublished
E1007	Isolate from feces of a non-hospitalized individual, identical to strain EnGen0015	B	61
E1679	Hospital outbreak (catheter tip)	A-2	61
E1636	Clinical isolate (bloodstream infection)	A-2	61
$\Delta chtS$	Markerless deletion mutant of <i>chtS</i> in E1162		This study
$\Delta chtR$	Markerless deletion mutant of <i>chtR</i> in E1162		This study
$\Delta chtS+chtS$	<i>In trans</i> complementation strain of $\Delta chtS$, $\Delta chtS$ harboring pEF25- <i>chtS</i>		This study
$\Delta chtR+chtR$	<i>In trans</i> complementation strain of $\Delta chtR$, $\Delta chtR$ harboring pEF25- <i>chtR</i>		This study
$\Delta chtS+pEF25$	Markerless deletion mutant of <i>chtS</i> in E1162 carrying empty pEF25		This study
$\Delta chtR+pEF25$	Markerless deletion mutant of <i>chtP</i> in E1162 carrying empty pEF25		This study
<i>E. coli</i>			
Ec1000	MC1000 <i>glgB::repA</i>		62
Plasmids			
pWS3	Gram-positive thermosensitive origin; Spc ^r		41
pWJ1	pWS3 derivative containing fused 5' and 3' flanking regions of <i>chtS</i>		This study
pWJ2	pWS3 derivative containing fused 5' and 3' flanking regions of <i>chtR</i>		This study
pWS3-Cre	Derivative of pWS3 expressing <i>cre</i> recombinase in <i>E. faecium</i>		31
pEF25	pAT18 derivative carrying spectinomycin cassette		63
pEF25- <i>chtS</i>	Plasmid for the complementation of $\Delta chtS$, pEF25 carrying <i>chtS</i>		This study
pEF25- <i>chtR</i>	Plasmid for the complementation of $\Delta chtR$, pEF25 carrying <i>chtR</i>		This study

Amp: ampicillin; Tet: tetracycline; Van: vancomycin; Spc: spectinomycin.

Table S2: Oligonucleotides used in this study

Primer	Sequence
Up-chtS_2202-F-XhoI	CCGCTCGAGGGAAGAAAACGGCTCCCG
Up-chtS_2202-R- EcoRI	GCGCAAACATCGAATTCGACGGCTGAATCAACTG
Down-chtS_2202-F- EcoRI	CCGTCGAATTCGATGTTGGCGCCAGCCAC
Down-chtS_2202-R-XmaI	CCCCCGGGCATTTCCAAATCGATGTACTG
Up-chtR_2203-F-XhoI	CCGCTCGAGGAAGCGGAAAGTGGCACTTTG
Up-chtR_2203-R- EcoRI	CAATTGATCGAATTC AACATCAATCGGTTACGCCG
Down-chtR_2203-F- EcoRI	CGATTGATGTTGGAATTCGATCAATTGTCGAATGATCTCTTCG
Down-chtR_2203-R-XmaI	CCCCCGGGATACATTAGCAATTAGAGAAAAGC
pAT392_EcoRI_lox66_genta_F	GAGGGAATTC TACCGTTCGATATAGCATA CATTATACGAAGTTATGATA
pAT392_EcoRI_lox71_genta_R	AACCCAGCGAACCATTTGAGG CTCCGAATTC TACCGTTCGATAAATGTATGCTATACGAAGTTATTCAAT CTTTATAAGTCCTTTTATAA
Comp2202_Fw_SacI	CCGGAGCTCTCCAAGCTAAGCCGGATAAA
Comp2202_Rv_SmaI	TTGCCCGGG AATGCATTACTTCGCCGTTT
Comp2203_Fw_SacI	CCGGAGCTCTGGACCCATGAGTCAATATAATC
Comp2203_Rv_SmaI	TTGCCCGGG ACCGTGAACACTCACCGATT

Restriction sites are underlined.

Supplemental references

3. **Lebreton F, van Schaik W, Manson McGuire A, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, Zeng Q, Wortman J, Birren B, Willems RJL, Earl AM, Gilmore MS.** 2013. Emergence of Epidemic Multidrug-Resistant *Enterococcus faecium* from Animal and Commensal Strains. *mBio* **4**:e00534-13-e00534-13.
31. **Zhang X, Paganelli FL, Bierschenk D, Kuipers A, Bonten MJM, Willems RJL, van Schaik W.** 2012. Genome-Wide Identification of Ampicillin Resistance Determinants in *Enterococcus faecium*. *PLoS Genet* **8**:e1002804.
40. **Zhang X, Vrijenhoek JEP, Bonten MJM, Willems RJL, van Schaik W.** 2011. A genetic element present on megaplasms allows *Enterococcus faecium* to use raffinose as carbon source. *Environ Microbiol* **13**:518–528.
59. **Lam MMC, Seemann T, Bulach DM, Gladman SL, Chen H, Haring V, Moore RJ, Ballard S, Grayson ML, Johnson PDR, Howden BP, Stinear TP.** 2012. Comparative analysis of the first complete *Enterococcus faecium* genome. *J Bacteriol* **194**:2334–2341.
60. **van Schaik W, Top J, Riley DR, Boekhorst J, Vrijenhoek JE, Schapendonk CM, Hendrickx AP, Nijman IJ, Bonten MJ, Tettelin H, Willems RJ.** 2010. Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island. *BMC Genomics* **11**:239.
61. **Leenhouts K, Buist G, Bolhuis A, ten Berge A, Kiel J, Mierau I, Dabrowska M, Venema G, Kok J.** 1996. A general system for generating unlabelled gene replacements in bacterial chromosomes. *Mol Gen Genet* **253**:217–224.

62. **Top J, Paganelli FL, Zhang X, van Schaik W, Leavis HL, van Luit-Asbroek M, van der Poll T, Leendertse M, Bonten MJM, Willems RJJ.** 2013. The *Enterococcus faecium* enterococcal biofilm regulator, EbrB, regulates the *esp* operon and is implicated in biofilm formation and intestinal colonization. PLOS One **8**:e65224.