

Supplementary Figures/Tables

Supplementary Figure S1

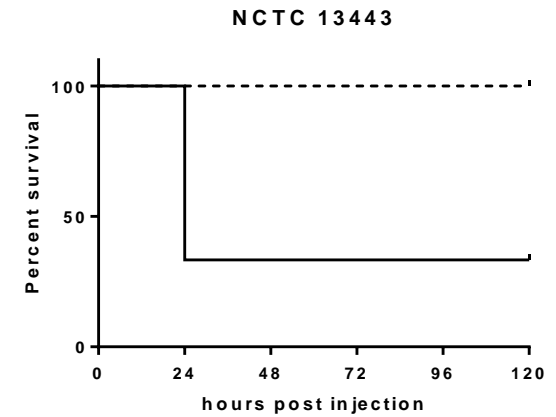
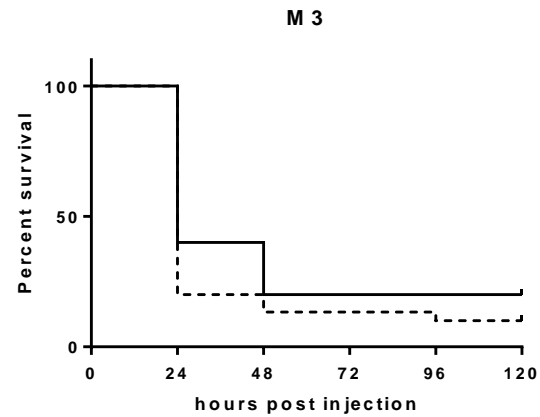
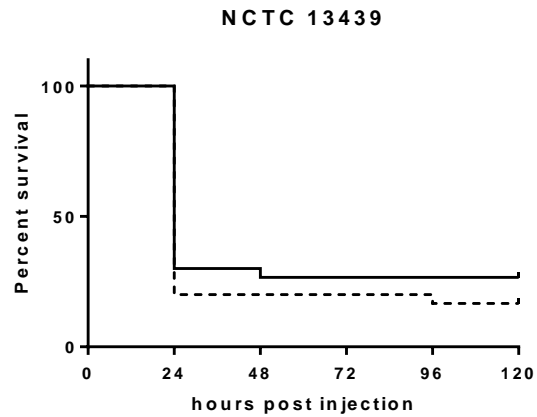
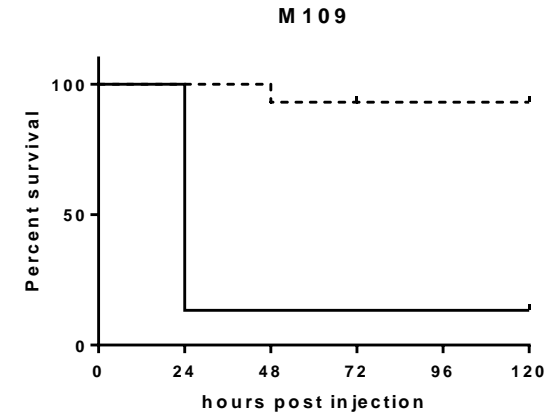
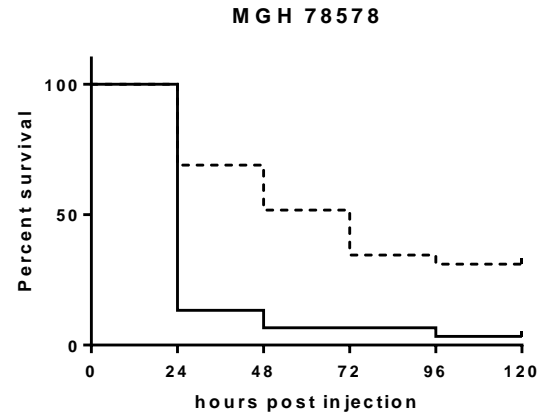
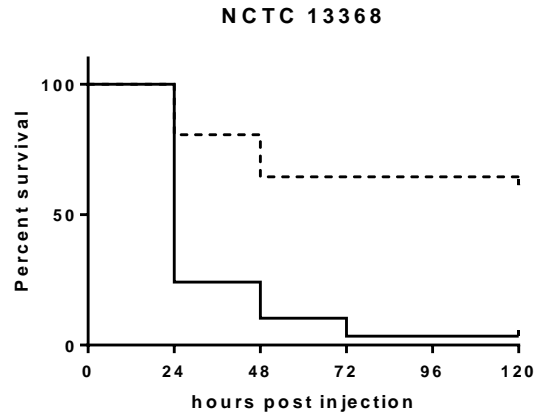


Figure S1. Virulence of *K. pneumoniae* strains in *Galleria* after adaptation to chlorhexidine. Groups of 30 larvae were challenged with 1×10^6 (NCTC 13443, MGH 78578); 1×10^5 (M109); 1×10^4 (NCTC 13368); 1×10^3 (M3, NCTC 13439) cfu. The number of alive vs. dead larvae was determined every 24 h post infection up to 120 h. Wild-type (Solid line) and chlorhexidine adapted (dashed line) are shown. Results were analysed using the Mantel-Cox method and highly significant differences in survival ($P < 0.0001$) were found for NCTC 13443, MGH 78578, M109 and NCTC 13368. No significant difference was found for M3 and NCTC 13439 ($P > 0.05$).

Table S1: Primers used in study

Gene	Primer name	F/R	Sequence (5'→3')	comment
qPCR Primers				
<i>rpoB</i>	LBOL064	F	AAATCACCCAAGGCGACGAT	
	LBOL065	R	ACCCTTGTTACCGTGACGAC	
<i>gapA</i>	LBOL066	F	GATGGCCCGTCTCACAAAGA	
	LBOL067	R	ATACCGGTCAGTTTGCCGTT	
<i>infB</i>	LBOL090	F	TTCTTCAAGTTTGCGGCGTG	
	LBOL091	R	CCCAGGCTGAAAAGATCCGT	
<i>phoP</i>	LBOL104			For all strains except M3 CA
		F	GCGAAGGATGGCAGGATAAAG	
	LBOL105 LBOL176	R	GCGGCAACCTCTTCAATATG	Replaces LBOL104 in M3 CA
<i>phoQ</i>	LBOL082	F	GCAAAGGATGGCAGGATAAAG	
	LBOL083	R	GCACGGAAACTTCCACGAAC	
<i>smvA</i>	LBOL162	F	GATCACTTTTGTGCGGCGAGC	
	LBOL163	R	CGCTGATGGCGATTTTGCTG	
<i>smvR</i>	LBOL062	F	CGCTGAGGGTTCGGCG	
	LBOL063	R	ATGATCGGCAGCGAGGATG	
<i>pmrK</i>	LBOL174	F	AGGTAGGCCGCTTTGATGTC	
	LBOL175	R	CACCGCGATGCTGGTGT	
<i>pmrD</i>	LBOL068			For all strains except 13368
		F	TGCTTCAGAGCGGTGCG	
	LBOL069 LBOL108	R	GCATCGGCCAGCGGAG	Replaces LBOL068 in 13368
<i>pagP</i>		F	CGTGCTTCAGAGCGGTACA	
	LBOL109	R	CGTGCTTCAGAGCGGTACA	
	LBOL120			For all strains except 13368
		R	CCGCTGACGGACCAGAATTT	
	LBOL121	F	GCCGGACCGTAACCTATTGA	
	LBOL122			Replaces LBOL120 in 13368
		R	CCGCTGACGGATCAGAACTT	
Plasmid complementation				
<i>phoPQ</i>	KPPhoProm			
	F ClaI	F	CTGAATCGATAATGGATAACCTGCG	
<i>phoPQ</i>	KPPhoQ R			
	XbaI	R	GCCGTCTAGATTAACCTCTGTTTATC	
<i>smvR</i>	K.p. cRP F			
	ClaI	F	CTTAATCGATGCTTAAAGCCGAT	
<i>smvR</i>	K.p. cRP R			
	XbaI	R	CGGATCTAGACCTAAAACAACCTCCAG	

Table S2. MICs of additional antibiotics for chlorhexidine-adapted strains. Antibiotics used were amikacin (AMK), ceftazidime (CAZ), ciprofloxacin (CIP), meropenem (MEM), chloramphenicol (CHL) and piperacillin/tazobactam (TZP). All values are given in mg/L. All MICs are shown as the range from at least three independent experiments.

	AMK	CAZ	CIP	MEM	CHL	TZP
M109 WT	4-8	≤0.5	0.06-0.125	0.06-0.125	8	4
M109 CA	4	≤0.5	0.06-0.125	≤0.06	4-8	2-4
NCTC 13439 WT	4-8	>512	4-8	16-32	16-32	512
NCTC 13439 CA	4-8	512->512	0.25-0.5 ^a	16-32	16	512
M3 WT	128-256	256	0.25-0.5	0.06-0.125	4	64
M3 CA	64-128	256-512	0.25-0.5	0.06-0.25	4	16-32
NCTC 13443 WT	>512	>512	>64	64->64	>512	>512
NCTC 13443 CA	>512	>512	>64	1-2 ^a	64 ^a	>512
NCTC 13368 WT	4-8	256	0.25-0.5	0.125-0.25	32	16
NCTC 13368 CA	4-8	256	0.5-1	0.25-0.5	32	16
MGH 78578 WT	64	512->512	1	0.06-0.125	>512	16
MGH 78578 CA	128-256	1-2 ^a	4-8 ^a	0.06-0.125	>512	16

^a ≥ 4-fold increase or decrease in MIC for chlorhexidine-adapted strains (CA) relative to non-adapted strains (WT)

Table S3 – List of additional chromosomal genetic changes after exposure to chlorhexidine and repeated passage

Strain and gene name (if known)	Type of Change	Change	MGH 78578 equivalent based on (NC_009648.1)	Function
NCTC 13439				
	SNP	In promoter	KPN_RS14630	Lipoprotein involved in maintaining OM lipid asymmetry
M3				
	SNP (synonymous)		KPN_RS21100	lipase
	SNP	In promoter	KPN_RS13675	D-arabinitol 4-dehydrogenase
NCTC 13368				
	SNP	E404V	KPN_RS22260	Porin protein
	Deletion (C) after nucleotide 150	Truncation of 52 aa, normally 181 aa	KPN_RS25745	Porin
MGH 78578				
	SNP (synonymous)		KPN_RS04285	Urocanate hydratase
	SNP (synonymous)		KPN_RS04265	Acyl-CoA thioesterase
	SNP	I11F	KPN_RS02945	DNA-binding protein
	SNP (synonymous)		KPN_RS19805	Hypothetical protein
	SNP	G9C	KPN_RS02085	Cytochrome C oxidase subunit III
	SNP	R547H	KPN_RS02165	Hypothetical protein
	SNP	A265T	KPN_RS03145	ABC transporter substrate-binding protein
<i>asnB</i>	SNP (synonymous)		KPN_RS03730	Asparagine synthetase B
	SNP	D174G	KPN_RS03925	Succinate dehydrogenase flavoprotein subunit
<i>sdhA</i>	SNP	Y287C	KPN_RS13300	Membrane protein
	SNP (synonymous)		KPN_RS13185	MATE family multidrug exporter
	SNP (synonymous)		KPN_RS13070	Membrane protein
	SNP (synonymous)		KPN_RS13040	Alpha-amylase
<i>tyrP</i>	SNP	V142A	KPN_RS12965	Tyrosine transporter
	SNP (synonymous)		KPN_RS12455	SpoVR family protein

	SNP	A284V	KPN_RS12365	Threonine dehydratase
	SNP		Prom of KPN_RS12070	Transporter
	SNP	T143A	KPN_RS11985	Nitrate ABC transporter substrate-binding protein
	In (C)	Truncation of 282aa (normally 460aa)	KPN_RS11960	Invasin
<i>narZ</i>	SNP (synonymous)		KPN_RS11935	Nitrate reductase A subunit alpha
	SNP	Q337R	KPN_RS11025	MFS transporter
	SNP (synonymous)		KPN_RS10495	LysR family transcriptional regulator
	SNP	G97V	KPN_RS09770	Coenzyme PQQ synthesis protein E
	SNP	A12V	KPN_RS09680	Hypothetical protein
<i>nlpI</i>	SNP	G272N	KPN_RS18270	Cystathionine beta-synthase
	SNP		Prom of KPN_RS19345	Lipoprotein
	Del (C)	Truncation of 163aa (normally 303aa)	KPN_RS18280	LysR family transcriptional regulator
<i>cysJ</i>	SNP	M177V	KPN_RS16795	Hypothetical protein
	SNP	Q529R	KPN_RS16705	Sulfite reductase subunit alpha
	SNP	E129G	KPN_RS16695	Phosphoadenosine phosphosulfate reductase
	SNP	Y79C	KPN_RS16115	Ribonucleoside-diphosphate reductase class 1b subunit alpha
<i>raiA</i>	SNP	V66A	KPN_RS15940	Xylose isomerase
	SNP	D51N	KPN_RS15660	Translation inhibitor protein
	SNP	V512A	KPN_RS25295	2-oxoglutarate dehydrogenase E2
	SNP	STOP51W (causes extra 8 aa)	KPN_RS00370	Hypothetical protein
	SNP		Prom of KPN_RS00865	Hypothetical protein
	SNP	D19G	KPN_RS01005	1-deoxy-D-xylulose 5-phosphate reductoisomerase
<i>pvcC</i>	SNP	D253N	KPN_RS25780	Pyoverdin chromophore biosynthetic protein
	SNP	D202G	KPN_RS26100	Thymidine phosphorylase
	SNP	Y39C	KPN_RS00035	Sodium:alanine symporter

<i>tynA</i>	SNP	V397A	KPN_RS07870	Tyramine oxidase
	SNP (synonymous)		KPN_RS08215	Cysteine methyltransferase
	SNP	V488A	KPN_RS13970	PTS fructose transporter subunit IIBC
	SNP	V62A	KPN_RS14240	L-rhamnonate dehydratase
	SNP	-	Prom of KPN_RS14630	Lipoprotein
	SNP	R80C	KPN_RS06285	Hypothetical protein
	SNP	D177G	KPN_RS06590	PTS N'-diacetylchitobiose transporter subunit IIC
	SNP	V198A	KPN_RS06930	Peptide ABC transporter permease
	SNP	-	Between KPN_RS17355 and KPN_RS17350	
	SNP (synonymous)	-	KPN_RS17280	GHMP kinase
	SNP (synonymous)	-	KPN_RS17110	Cobalt-precorrin-2 C(20)-methyltransferase
	SNP	K368R	KPN_RS21675	Alpha-glucosidase
	SNP	T102A	KPN_RS05660	Molecular chaperone
	SNP	N58S	KPN_RS05955	Transporter
SNP	Q140STOP (normally 313 aa)	KPN_RS08600	DNA binding protein	
SNP (synonymous)	-	KPN_RS08515	D-mannonate oxidoreductase	
<i>fusA</i>	SNP	R309C	KPN_RS20135	Elongation factor G
	SNP	V263A	KPN_RS20465	Transcriptional regulator
	SNP	I322V	KPN_RS20520	1,4-alpha-glucan-branching protein
	SNP	P251S	KPN_RS20535	Gluconate transporter
	SNP	N164D	KPN_RS20545	Transcriptional regulator
	SNP (synonymous)	-	KPN_RS15195	Phosphatase
	SNP	Y1096C	KPN_RS15460	Phosphoribosylformylglycinamide synthase

	SNP	S69G	KPN_RS15390	Membrane protein
	Del (G)	Truncation to 31aa (normally 212aa)	KPN_RS06250	Leucine efflux protein
	Del (C)	Truncation to 74aa (normally 357aa)	KPN_RS17010	Hypothetical protein
<i>wza</i>	Del (G)	Truncation to 92aa (normally 377aa)	KPN_RS13510	Polysaccharide export protein
