

Table 1. Bacterial strains used and constructed in this study

Strain	Description	Reference or source
<i>K. pneumoniae</i>		
CG43S3	CG43 Sm ^r	Laboratory stock
U9451	CG43S3 <i>galU</i> deletion mutant	Laboratory stock
LacZ16	CG43S3 <i>lacZ</i> Sm ^r	Laboratory stock
<i>kvhA</i> ⁻	LacZ16 <i>kvhA</i> Sm ^r	Laboratory stock
<i>kvgA</i> ⁻	LacZ16 <i>kvgA</i> Sm ^r	Laboratory stock
<i>kvhR</i> ⁻	LacZ16 <i>kvhR</i> Sm ^r	Laboratory stock
<i>kvhA</i> ⁻ <i>kvgA</i> ⁻	LacZ16 <i>kvhA</i> <i>kvgA</i> Sm ^r	This study
<i>kvhA</i> ⁻ <i>kvhR</i> ⁻	LacZ16 <i>kvhA</i> <i>kvhR</i> Sm ^r	This study
<i>kvgA</i> ⁻ <i>kvhR</i> ⁻	LacZ16 <i>kvgA</i> <i>kvhR</i> Sm ^r	This study
<i>kvhA</i> ⁻ <i>kvgA</i> ⁻ <i>kvhR</i> ⁻	LacZ16 <i>kvhA</i> <i>kvgA</i> <i>kvhR</i> Sm ^r	This study
<i>rpoS</i> ⁻	LacZ16 <i>rpoS::Tc</i> Sm ^r	Laboratory stock
<i>rpoS</i> ⁻ <i>kvhA</i> ⁻	LacZ16 <i>rpoS::Tc</i> <i>kvhA</i> Sm ^r	This study
<i>phoP</i> ⁻	LacZ16 <i>phoP</i> Sm ^r	Laboratory stock
<i>E. coli</i>		
S17-1 λ <i>pir</i>	<i>hsdR</i> <i>recA</i> <i>pro</i> RP4-2 (Tc::Mu; Km::Tn7)(λ <i>pir</i>)	De Lorenzo <i>et al.</i> , 1994
JM109	<i>RecA1</i> <i>supE44</i> <i>endA1</i> <i>hsdR17</i> <i>gyrA96</i> <i>rolA1</i> <i>thi</i> (<i>lac-proAB</i>)	Laboratory stock
BL21-RIL (DE3)	F ⁻ <i>ompT</i> <i>hsdS_B</i> (<i>r_B</i> - <i>m_B</i> -) <i>gal</i> <i>dcm</i> (DE3)	Laboratory stock

Table 2. Plasmids used and constructed in this study

Plasmid	Description	Reference or source
pGEM-T Easy	PCR cloning vector, Ap ^r	Promega
yT&A	PCR cloning vector, Ap ^r	Sigma
pRK415	Shuttle vector, <i>mob</i> ⁺ Tc ^r	Laboratory stock
placZ15	A derivative of pYC016, containing <i>lacZ</i> as a reporter, Cm ^r	Laboratory stock
pA13	pKAS46 carrying a <i>kvgA</i> fragment	Laboratory stock
pR14	pKAS46 carrying a <i>kvhR</i> fragment	Laboratory stock
pRpoS46	pKAS46 carrying <i>rpoS::Tc</i> fragment	Laboratory stock
pkvhrP-1	A 470 bp PCR product of <i>kvhR</i> promoter region cloned into pUC-T	Laboratory stock
pHP4005	pET30C carrying the entire <i>kvhA</i> coding sequence	Laboratory stock
pAG	A 1.3 kb PCR product of the <i>kvhA</i> locus with the putative promoter cloned into pGEM-T Easy	This study
pA415	An <i>EcoRI</i> fragment of pAG cloned into the <i>EcoRI</i> site of pRK415	This study
pA15	A <i>BamHI</i> fragment of pA cloned into <i>BamHI</i> site of placZ15	Laboratory stock
pEG	A 300 bp PCR product of <i>kvhA</i> promoter region cloned into pGEM-T Easy	This study
pE15	A <i>BamHI/BglIII</i> fragment of pEG cloned into <i>BamHI</i> site of placZ15	This study
pFY	A 450 bp PCR product of <i>kvhA</i> promoter region cloned into yT&A	This study
pF15	A <i>BamHI/BglIII</i> fragment of pFY cloned into <i>BamHI</i> site of placZ15	This study

pETm-c	A derivative of pET30C, containing malonate promoter, Km ^r	Laboratory stock
pHAm-c	A <i>Bam</i> HI fragment of pHP4005 carrying entire <i>kvhA</i> coding sequence cloned into the <i>Bam</i> HI site of pETm-c	This study
pHANY	A 460 bp PCR product of <i>kvhA</i> without HTH motif sequence cloned into yT&A	This study
pHANm-c	A <i>Bam</i> HI fragment of pHANY cloned into the <i>Bam</i> HI site of pETm-c	This study
porf1Y	A 800 bp PCR product of <i>cps orf1-2</i> promoter region cloned into yT&A	This study
porf2Y	A 440 bp PCR product of <i>cps orf1-2</i> promoter region cloned into yT&A	This study
porf3Y	A 950 bp PCR product of <i>cps porf3-15</i> promoter region cloned into yT&A	This study
porf4Y	A 500 bp PCR product of <i>cps porf3-15</i> promoter region cloned into yT&A	This study
porf162Y	A 400 bp PCR product of <i>cps porf16-17</i> promoter region cloned into yT&A	This study
porf1Z15	A <i>Bam</i> HI fragment of porf1Y cloned into the <i>Bam</i> HI site of placZ15	This study
porf2Z15	A <i>Bgl</i> III fragment of porf2Y cloned into the <i>Bam</i> HI site of placZ15	This study
porf3Z15	A <i>Bam</i> HI fragment of porf3Y cloned into the <i>Bam</i> HI site of placZ15	This study
porf4Z15	A <i>Bgl</i> III fragment of porf4Y cloned into the <i>Bam</i> HI site of placZ15	This study
porf162Z15	A <i>Bgl</i> III fragment of porf162Y cloned into the <i>Bam</i> HI site of placZ15	This study

Table 3. Primers used in this study

Primer	Sequences	Restriction site design
A201	5'-GTGAAAAAGCTTCGTTCA-3'	
AS02	5'-CAGCCATGCTTTCTCCTT-3'	
AS07	5'-ATCAGGATCCACGCCCC-3'	5'- <i>Bam</i> HI
AP01	5'-GAACGCCGGATCCTACAGC-3'	5'- <i>Bam</i> HI
AP02	5'-GCTGTCGAGATCTGCCGC-3'	5'- <i>Bgl</i> III
AP04	5'-CATCAGATGGATCCAAACCC-3'	5'- <i>Bam</i> HI
HA01	5'-TGC GTTGAAAGCTTATCCAG-3'	5'- <i>Hind</i> III
P040	5'-ACTGGATCCAGGCCTGGTAATAGCCATT-3'	5'- <i>Bam</i> HI
P041	5'-ACTGGATCCCGCTGTCGTATCTCAATG-3'	5'- <i>Bam</i> HI
P046	5'-ACTGGATCCAGACGGAGGAACTGTTTC-3'	5'- <i>Bam</i> HI
P074	5'-ACTGGATCCACGATCATGGATAAGAT-3'	5'- <i>Bam</i> HI
P075	5'-ACTGGATCCTGCGACCGGAATAACC-3'	5'- <i>Bam</i> HI
PCPS1	5'-GGGCAGATCTCAGTTCACCG-3'	5'- <i>Bgl</i> III
PCPS2	5'-GGTGCGCAGATCTATAAGC-3'	5'- <i>Bgl</i> III
PCPS3	5'-GGCAGATCTTCGGTAACAAC-3'	5'- <i>Bgl</i> III

Table 4. CPS synthesis of the LacZ16-derived mutants

Strains	Mean quantity \pm SD ^a	Muroid phenotype ^b
LacZ16	22.8 \pm 3.8	+ +
kvhA ⁻	24.4 \pm 2.4	+ +
kvhR ⁻	13.6 \pm 1.5	-
kvhA ⁻ kvhR ⁻	17.6 \pm 0.9	+
kvgA ⁻	15.7 \pm 0.3	-
kvhA ⁻ kvgA ⁻	11.9 \pm 2.2	-
kvgA ⁻ kvhR ⁻	12.9 \pm 0.8	-
kvhA ⁻ kvgA ⁻ kvhR ⁻	16.1 \pm 0.3	-

^a Values are the averages of triplicate samples and are given as micrograms of uronic acid per 10⁹ CFU.

^b Assessed by string formation test after 48 h grown on LB medium. Symbols: - , negative; + , positive; + + , strong.

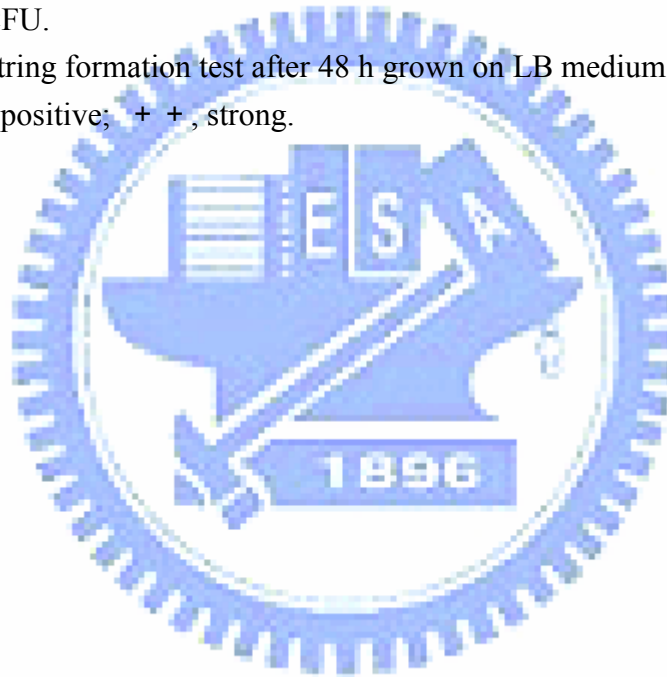


Table 5. Virulence properties of the mutants derived from *K. pneumoniae* LacZ16

Strain	LD ₅₀ (CFU)	Survival rate in NHS (%) ^a	Survival rate in HI-NHS (%) ^a
LacZ16	3×10 ³	>95	>95
kvhA-	3×10 ³	>85	>85
kvhR-	>3×10 ⁵	>85	>95
kvhA-kvhR-	>3×10 ⁵	>20	>50
kvhA-kvhR-[pA415]	>3×10 ⁵	≈40	>80
kvgA-	5×10 ⁴ ~5×10 ⁵	>80	>90
kvhA-kvgA-	>4×10 ⁵	>80	>80
kvgA-kvhR-	>4×10 ⁵	>80	>80
kvhA-kvgA-kvhR-	>4×10 ⁵	>75	>75
CG43S3	-	>90	>95
U9451(control) ^b	-	0	>60

^a Percentage of survival rate in human serum is expressed as 100× (the number of viable bacteria after treatment / the number of viable bacteria before treatment). NHS: nonimmune human sera; HI-NHS: heat-inactivated human sera.

^b U9451, a *galU* mutant strain with defective CPS and lipopolysaccharide.

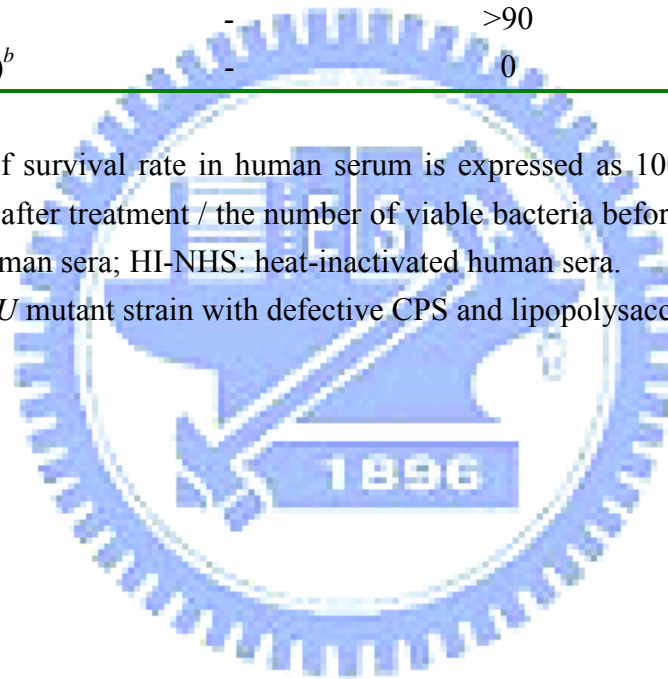


Table 6. Effect of KvhA overexpression in *K. pneumoniae* CG43S3 on the resistance activity to antibiotics.

Antibiotic ($\mu\text{g}/\text{disk}$)	Zone (mm) ^a / <i>K. pneumoniae</i> CG43S3	Zone (mm) / Overexpression of KvhA	Zone (mm) / Overexpression of KvhA* ^b
-Lactams (PBP inhibitors)			
ceftazidime 30	24	24	24
cefamandole 30	21	21	21
cefotaxime 30	24	24	24
cephalothin 30	20	13	20
methicillin 5	0	0	0
penicillin 10	0	0	0
piperacillin 100	20	13	20
ticarcillin 75	10	0	10
carbenicillin 100	11	0	11
Other cell wall inhibitor			
fosfomycin 50	17	28	17
Protein synthesis inhibitor			
erythromycin 15	0	0	0
DNA synthesis inhibitor			
novobiocin 5	0	0	0
nalidixic acid 30	20	20	20
Metabolism inhibitor			
trimethoprim 1.25 + sulfamethoxazole 23.75	18	22	18
Cell membrane disruption			
polymyxin B 300	12	12	12

^a Diameter of zones of inhibition, measured across disks of 6 mm diameter. Antibiotics that did not inhibit growth of the bacterial lawn were assigned a value of 0 mm.

^b The KvhA without HTH motif.