

Table S1. Pneumococcal strains used in the study

Strain	Identifyer	Genotype	Reference	WGS	Fig. ^a
D39	BHN853	D39, wildtype, Serotype 2, (parental strain for BHN857-BHN860 and BHN870)	Laboratory collection	WGS	
D39- <i>fakB3</i> -mutant_1	BHN857	Spontaneous 2CCA-1 resistant D39 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	S3
D39- <i>fakB3</i> -mutant_2	BHN858	Spontaneous 2CCA-1 resistant D39 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
D39- <i>fabT</i> -mutant_1	BHN859	Spontaneous 2CCA-1 resistant D39 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fabT</i>	This study	WGS	S2
D39- <i>fakB3</i> -mutant_3	BHN860	Spontaneous 2CCA-1 resistant D39 strain isolated after overnight exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	S3
D39- <i>fakB3</i> -mutant_4	BHN870	Spontaneous 2CCA-1 resistant D39 strain isolated after overnight exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
D39Δ <i>fakB3</i> :: <i>ermB</i>	BHN2024	Strain BHN853 with Orf <i>SPD0646</i> encoding “FakB3” replaced with an <i>ermB</i> cassette	This study		S3
BHN857Δ <i>fakB3</i> :: <i>ermB</i>	BHN2025	Strain BHN857 with Orf <i>SPD0646</i> encoding “FakB3” replaced with an <i>ermB</i> cassette	This study		S3
D39(<i>SPD0645</i> -∇ <i>ermB</i> - <i>SP0646</i>)	BHN2027	Strain BHN853 with <i>ermB</i> cassette inserted between <i>fakB3</i> (<i>SPD0646</i>) and (<i>SPD0645</i>)	This study		S3
BHN857∇ <i>fakB3</i> -(<i>SPD0645</i> -∇ <i>ermB</i> - <i>SP0646</i>)	BHN2028	Mutant <i>fakB3</i> allele of BHN857 replaced with a wildtype <i>fakB3</i> allele and with an <i>ermB</i> cassette inserted between <i>fakB3</i> (<i>SPD0646</i>) and (<i>SPD0645</i>)	This study		S3
D39Δ <i>fakB1</i> :: <i>kanR</i>	BHN2030	<i>fakB1</i> (<i>SPD1388</i>) Orf replaced with a kanamycin resistance cassette	This study		
D39Δ <i>fakB2</i> :: <i>spcR</i>	BHN2031	<i>fakB2</i> (<i>SPD0996</i>) Orf replaced with a spectinomycin resistance cassette	This study		
D39Δ <i>fakB1</i> :: <i>ermB</i> Δ <i>fakB2</i> :: <i>tetR</i>	BHN1351	<i>fakB1</i> (<i>SPD1388</i>) Orf replaced with an erythromycin resistance Orf; <i>fakB2</i> (<i>SPD0996</i>) Orf replaced with a tetracyclin resistance Orf	This study		
D39Δ <i>fabT</i> :: <i>ermB</i>	BHN2032	Orf <i>SPD0379</i> encoding “FabT” replaced with an <i>ermB</i> Orf	This study		S2
D39(<i>SPD0379</i> -∇ <i>ermB</i> - <i>SPD0380</i>)	BHN2022	<i>ermB</i> Orf inserted between <i>fabT</i> (<i>SPD0379</i>) and <i>fabH</i> (<i>SPD0380</i>)	This study		S2
BHN859∇ <i>fabT</i> -(<i>SPD0379</i> -∇ <i>ermB</i> - <i>SPD0380</i>)	BHN2023	Mutant <i>fabT</i> allele of BHN859 replaced with a wildtype <i>fabT</i> allele and with an <i>ermB</i> Orf inserted between <i>fabT</i> (<i>SPD0379</i>) and <i>fabH</i> (<i>SPD0380</i>)	This study		S2
D39Δ <i>lytA</i> :: <i>ermB</i>	BHN2042	Orf <i>SPD1737</i> encoding “LytA” replaced with an <i>ermB</i> cassette	This study		

Strain	Identifyer	Genotype	Reference	WGS	Fig. ^a
Tigr4 (T4)	BHN842	Tigr4, wildtype, Serotype 4 (parental strain for BHN848-BHN852)	Laboratory collection	WGS	
T4- <i>fabT</i> -mutant_1	BHN848	Spontaneous 2CCA-1 resistant Tigr4 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fabT</i>	This study	WGS	S2
T4- <i>fakB3</i> -mutant_1	BHN849	Spontaneous 2CCA-1 resistant Tigr4 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
T4- <i>fakB3</i> -mutant_2	BHN850	Spontaneous 2CCA-1 resistant Tigr4 strain isolated after 2 hours exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
T4- <i>fakB3</i> -mutant_3	BHN851	Spontaneous 2CCA-1 resistant Tigr4 strain isolated after overnight exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
T4- <i>fakB3</i> -mutant_4	BHN852	Spontaneous 2CCA-1 resistant Tigr4 strain isolated after overnight exposure to 2CCA-1, includes a mutation in <i>fakB3</i>	This study	WGS	
T4Δ <i>fakB3</i> :: <i>ermB</i>	BHN2033	Orf SP0742 encoding “FakB3” replaced with an <i>ermB</i> cassette	This study		S3
T4Δ <i>fabT</i> :: <i>ermB</i>	BHN2034	Orf SP0416 encoding “FabT” replaced with an <i>ermB</i> Orf	This study		S2
T4(<i>SP0416</i> -∇ <i>ermB</i> - <i>SP0417</i>)	BHN2035	<i>ermB</i> Orf inserted between <i>fabT</i> (<i>SP0416</i>) and <i>fabH</i> (<i>SP0417</i>)	This study		S2
BHN848∇ <i>fabT</i> -(<i>SP0416</i> -∇ <i>ermB</i> - <i>SP0417</i>)	BHN2036	Mutant <i>fabT</i> allele of BHN848 replaced with a wildtype <i>fabT</i> allele and with an <i>ermB</i> Orf inserted between <i>fabT</i> (<i>SP0416</i>) and <i>fabH</i> (<i>SP0417</i>)	This study		S2
T4Δ <i>SP0743</i> :: <i>ermB</i>	BHN2037	Orf <i>SP0743</i> encoding “transcriptional regulator, TetR family”, replaced with an <i>ermB</i> cassette	This study		S3
T4Δ <i>SP0741</i> :: <i>ermB</i>	BHN2038	Orf <i>SP0741</i> encodes “NAD(P)/FAD-dependent oxidoreductase”, replaced with an <i>ermB</i> cassette	This study		S3
T4Δ <i>SP0740</i> :: <i>ermB</i>	BHN2039	Orf <i>SP0740</i> encodes “MutT/NUDIX family protein”, replaced with an <i>ermB</i> cassette	This study		S3
T4Δ <i>SP0740-SP0742</i> :: <i>ermB</i>	BHN2040	Orfs <i>SP0740-SP0742</i> replaced with an <i>ermB</i> cassette	This study		S3

Notes: ^a) See indicated Supplementary figure for extended information about the strain.