

Effect	increased activity	no effect	intermediate effect	100 % inhibition
Comments	activity higher as wildtype AcrB	behavior like wildtype; for MICs including one MIC value less than wildtype AcrB	behavior between wildtype and negative control; for MICs in the range of two MIC values less than wildtype and two MIC values higher than negative control	behavior like negative control or dead mutant; for MICs within two MIC values higher than negative control

Position	Area	Mutation	Methods	Substrate	Genome-based strains		Plasmid-based strains		Expression level	Structure available	PMID											
					Comp to pos ctrl	Comp to neg ctrl	Comp to pos ctrl	Comp to neg ctrl														
N3	Cytoplasm; N-terminal	CysLess: N3C Cys was replaced by Ala	MIC	Novobiocin	/	/	Increased activity 200%	/	/	/	11754746											
W13	TM1/TM6/TM8	W13C_C493A_C887A	MIC	Erythromycin	/	/	No effect	/	/	/	24854514											
				Novobiocin	/	/																
				Rhodamine 6G	/	/																
A22	TM1	A22P_T85A	MIC	TPP	/	/	/	/	/	/	27161641											
				Linezolid	No effect	/																
				Chloramphenicol	Intermediate effect (~25% of WT activity)	4x activity of neg ctrl																
				Cefuroxime	No effect	/																
				Oxacillin																		
				Clindamycin																		
				Tetracycline																		
				Tigecycline																		
				Levofloxacin																		
				Novobiocin																		
				Rifampicin																		
				Clarithromycin								Increased MIC (400% activity)	/									
				Erythromycin								No effect	/									
Azithromycin	No effect	/																				
L28	TM1	L28F_G141D_L226T_N282Y	MIC	Levofloxacin	/	/	/	/	/	/	25182653											
				Chloramphenicol																		
				Oxacillin																		
				Linezolid																		
				Minocycline																		
				Novobiocin																		
			Clarithromycin																			
			Rifampicin																			
			Levofloxacin	No effect	/																	
			Chloramphenicol																			
			Oxacillin																			
			Linezolid			Intermediate effect (~12% of WT activity)						activity of neg ctrl (Δ Ad)										
			Minocycline																			
Novobiocin																						
Clarithromycin																						
K29	In between TM1 and PN1; central cavity	K29A	MIC	Novobiocin	/	/	No effect	/	comparable to WT	/	16166543											
				Tetracycline																		
				Norfloxacin																		
				Erythromycin																		
				Taurocholic acid																		
				Ethidium																		
				Dequalinium																		
				Rhodamin-6-G																		
				Acriflavine																		
				SDS																		
				P31								between TM1 and PN1	P31A	Complementation assay	Ciprofloxacin	/	/	No effect	Higher growth	Comparable to WT	/	28497315
													P31G									
				V32								between TM1 and PN1	CysLess: V32C Cys was replaced by Ala	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659
Rhodamine 6G																						
Berberine																						
Erythromycin																						
Oxacillin																						
V32: between TM1 and PN1_N298;PN2; TM1-PN2 interface	CysLess: V32C_N298C Cys was replaced by Ala	MIC	Tetraphenylphosphonium		/	/	Intermediate effect (~6% of WT activity)	8x activity of neg ctrl (empty vector)														
			Rhodamine 6G						Intermediate effect (~12% of WT activity)	2x activity of neg ctrl (empty vector)												
			Berberine						Intermediate effect (~12% of WT activity)	4x activity of neg ctrl (empty vector)												
			Erythromycin						Intermediate effect (~25% of WT activity)	8x activity of neg ctrl (empty vector)												
V32: between TM1 and PN1_A299;PN2; TM1-PN2 interface	CysLess: V32C_A299C Cys was replaced by Ala	MIC	Tetraphenylphosphonium		/	/	No effect	/														
			Rhodamine 6G																			
			Berberine																			
			Erythromycin																			
V32: between TM1 and PN1_I390; between TM3 and TM4	CysLess: V32C_I390C Cys was replaced by Ala	MIC	Erythromycin	/	/	No effect	/	Comparable to WT	/	21529338												
			Novobiocin																			
between TM1 and PN1	V32P	Complementation assay	Ciprofloxacin	/	/	No effect	Higher growth	Comparable to	/	28497315												
			A33P	No effect	Higher growth																	
A33: between TM1 and PN1_I390;	A33P_I390P	Complementation assay	Ciprofloxacin	/	/	100% inhibition	like dead mutant	Comparable to WT	/	28497315												
			A33P_T392P																			

A33	A33_T37: between TM1 and PN1	A33W_T37W		Benzalkonium chloride Rhodamine 6G Ethidium bromide Acriflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	No effect	/				
	A33_T37: between TM1 and PN1_T298: PN2	A33W_T37W_N298W	MIC on agar plates	Benzalkonium chloride Rhodamine 6G Ethidium bromide Acriflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	Intermediate effect (~12% of WT activity) Intermediate effect (~3% of WT activity) Intermediate effect (~12% of WT activity) Intermediate effect (~3% of WT activity) No effect	8x activity of neg ctrl (ΔacrB) 16x activity of neg ctrl (ΔacrB) 2x activity of neg ctrl (ΔacrB) /	Comparable to WT	Residue was identified using PDB 3AOD	29317622	
	A33_T37: between TM1 and PN1_A100: PN1_T298: PN2	A33W_T37W_A100W_N298W		Benzalkonium chloride Rhodamine 6G Ethidium bromide Acriflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	Intermediate effect (~25% of WT activity) Intermediate effect (~6% of WT activity) No effect Intermediate effect (~6% of WT activity) No effect	16x activity of neg ctrl (ΔacrB) 16x activity of neg ctrl (ΔacrB) / 4x activity of neg ctrl (ΔacrB) /				
				Benzalkonium chloride Rhodamine 6G Ethidium bromide Acriflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	Intermediate effect (~12% of WT activity) 100% inhibition Intermediate effect (~12% of WT activity) Intermediate effect (~25% of WT activity) No effect	32x activity of neg ctrl (ΔacrB) 16x activity of neg ctrl (ΔacrB) / 32x activity of neg ctrl (ΔacrB) 16x activity of neg ctrl (ΔacrB) /				
	Y35	between TM1 and PN1	Y35N	MIC	Linezolid Chloramphenicol Cefuroxime Oxacillin Clindamycin Tetracycline Tigecycline Levofloxacin Novobiocin Rifampicin Clarithromycin Erythromycin Azithromycin	No effect	/	/	/	/	/	27161641
	P36	between TM1 and PN1	P36A P36G	Complementation assay	Ciprofloxacin	/	/	No effect	Higher growth	Comparable to WT	/	28497315
	T37	between TM1 and PN1	T37W	MIC on agar plates	Benzalkonium chloride Rhodamine 6G Ethidium bromide Acriflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	No effect	/	Comparable to WT	Residue was identified using PDB 3AOD	29317622
			I38F	MIC	Linezolid Chloramphenicol Cefuroxime Oxacillin Clindamycin Tetracycline Tigecycline Levofloxacin Novobiocin Rifampicin Clarithromycin Erythromycin Azithromycin Ethidium	Intermediate effect (~12% of WT activity) Intermediate effect (~12% of WT activity) Intermediate effect (~6% of WT activity) Intermediate effect (~25% of WT activity) No effect Intermediate effect (~25% of WT activity) No effect No effect Increased MIC (400%) No effect	activity of neg ctrl (ΔAc) activity of neg ctrl (ΔAc) activity of neg ctrl (ΔAc) activity of neg ctrl (ΔAc) / activity of neg ctrl (ΔAc) / / / /	Intermediate effect (~12% of WT activity) 100% inhibition Intermediate effect (~25% of WT activity) Intermediate effect (~12% of WT activity) No effect Increased MIC (200%) No effect	2x activity of neg ctrl (ΔAcB) / 32x activity of neg ctrl (ΔAcB) 8x activity of neg ctrl (ΔAcB) / / /			

I38

PN1 (I38) bottom of the proximal binding pocket

	% dye accumulation	Hoechst 33342	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Pyronin Y	Increased accumulation comp to WT	Decreased accumulation comp	/	/
	Efflux assay	Hoechst 33342	No effect	/	No effect	/
		Berberine	/	/	/	/
I38F_1671T	MIC	Pyronin Y	100% inhibition	/	/	/
		Nile Red	/	/	No effect	/
		1,2-DINA	/	/	No effect	/
		Linezolid	100% inhibition	/	/	/
		Chloramphenicol	100% inhibition	/	/	/
		Cefuroxime	Intermediate effect (~1.5% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Oxacillin	Intermediate effect (~3% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Clindamycin	100% inhibition	/	/	/
		Tetracycline	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Tigecycline	100% inhibition	/	/	/
		Levofloxacin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Novobiocin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Rifampicin	No effect	/	/	/
		Clarithromycin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Erythromycin	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Azithromycin	Intermediate effect (<1% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Tedizolid	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Minocycline	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Moxifloxacin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Ciprofloxacin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Rifaximin	Increased MIC (not further specified)	/	/	/
		Oleandomycin	No effect	/	/	/
		Tylosin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Josamycin	No effect	/	/	/
		Ethidium	No effect	/	/	/
		Hoechst 33342	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Berberine	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Pyronin Y	No effect	/	/	/
Ethidium	Increased accumulation comp to WT	Decreased accumulation comp	/	/		
Hoechst 33342	100% inhibition	/	/	/		
Berberine	/	/	No effect	/		
Pyronin Y	/	/	No effect	/		
Nile Red	/	/	No effect	/		
1,2-DINA	/	/	No effect	/		
I38F_A103T_A160T	MIC	Linezolid	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Chloramphenicol	100% inhibition	/	/	/
		Cefuroxime	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Oxacillin	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Clindamycin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Tetracycline	100% inhibition	Equal activity	/	/
		Tigecycline	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Levofloxacin	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Novobiocin	No effect	/	/	/
		Rifampicin	No effect	/	/	/
		Clarithromycin	No effect	/	/	/
		Erythromycin	No effect	/	/	/
		Azithromycin	No effect	/	/	/
		I38F_R259S	MIC	Linezolid	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)
Chloramphenicol	100% inhibition			Equal activity	/	/
Cefuroxime	Intermediate effect (~6% of WT activity)			activity of neg ctrl (ΔAc)	/	/
Oxacillin	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)	/	/
Clindamycin	No effect			/	/	/
Tetracycline	100% inhibition			Equal activity	/	/
Tigecycline	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)	/	/
Levofloxacin	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)	/	/
Novobiocin	No effect			/	/	/
Rifampicin	No effect			/	/	/
Clarithromycin	Increased MIC (400%)			/	/	/
Erythromycin	No effect			/	/	/
Azithromycin	No effect			/	/	/
I38F_N144S_A209E	MIC			Linezolid	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)
		Chloramphenicol	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Cefuroxime	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Oxacillin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Clindamycin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Tetracycline	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Tigecycline	No effect	/	/	/
		Levofloxacin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)	/	/
		Novobiocin	No effect	/	/	/
		Rifampicin	No effect	/	/	/
		Clarithromycin	Increased MIC (400%)	/	/	/
		Erythromycin	No effect	/	/	/
		Azithromycin	No effect	/	/	/

27161641

		I38F_P50L_T196M	MIC	Linezolid	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)	/	/												
				Chloramphenicol	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)														
				Cefuroxime	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)														
				Oxacillin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)														
				Clindamycin	No effect	/														
				Tetracycline	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)														
				Tigecycline																
				Levofloxacin	Intermediate effect (~25% of WT activity)	activity of neg ctrl (ΔAc)														
				Novobiocin	No effect	/														
				Rifampicin																
				Clarithromycin	Increased MIC (400%)	/														
				Erythromycin	No effect	/														
				Azithromycin																
				I38N_G147D	MIC	Linezolid						Intermediate effect (~3% of WT activity)	activity of neg ctrl (ΔAc)	/	/					
						Chloramphenicol						100% inhibition	Equal activity							
		Cefuroxime	Intermediate effect (~6% of WT activity)			activity of neg ctrl (ΔAc)														
		Oxacillin	Intermediate effect (~12% of WT activity)			activity of neg ctrl (ΔAc)														
		Clindamycin	Intermediate effect (~12% of WT activity)			activity of neg ctrl (ΔAc)														
		Tetracycline	100% inhibition			Equal activity														
		Tigecycline																		
		Levofloxacin	Intermediate effect (~12% of WT activity)			activity of neg ctrl (ΔAc)														
		Novobiocin	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)														
		Rifampicin																		
		Clarithromycin	No effect			/														
		Erythromycin																		
		Azithromycin																		
		I38S_P116L_A294V	MIC			Linezolid	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)	/	/										
						Chloramphenicol	100% inhibition	Equal activity												
				Cefuroxime	Intermediate effect (~6% of WT activity)	activity of neg ctrl (ΔAc)														
				Oxacillin	Intermediate effect (~12% of WT activity)	activity of neg ctrl (ΔAc)														
Clindamycin	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)																
Tetracycline	100% inhibition			Equal activity																
Tigecycline	No effect			/																
Levofloxacin	Intermediate effect (~25% of WT activity)			activity of neg ctrl (ΔAc)																
Novobiocin																				
Rifampicin																				
Clarithromycin	No effect			/																
Erythromycin																				
Azithromycin																				
A39	PN1			A39T	MIC	Linezolid	No effect	/						/	/	/	/	27161642		
						Chloramphenicol														
		Cefuroxime																		
		Oxacillin																		
		Clindamycin																		
		Tetracycline																		
		Tigecycline																		
		Levofloxacin																		
		Novobiocin																		
		Rifampicin																		
		Clarithromycin	Increased MIC (400%)																	
		Erythromycin	No effect																	
		Azithromycin																		
		CysLess: A39C Cys was replaced by Ala	MIC			Novobiocin			/	/	No effect	/	/						/	11754746
		T44	T44: PN1_T91: PN1			CysLess: T44C_T91C Cys was replaced by Ala			MIC	Erythromycin	/	/	No effect						/	Comparable to WT
Y49	PN1	Y49S	MIC	Erythromycin	/	/	No effect	/	/	/	26240069									
				Novobiocin																
				Minocycline																
				Nalidixic acid																
S79	PN1	S79R_T91R	Growth inhibition	Erythromycin	/	/	Decreased growth compared to WT	Growth slightly higher comp to neg ctrl (empty vector)	Comparable to WT	/	22121023									
			accumulation Assay of Doxorubicin	None; only Doxorubicin	/	/	No effect	/												
				Erythromycin	/	/	Intermediate effect: no inhibition of doxo efflux	Slightly higher doxo efflux comp to neg ctrl (empty vector)												
T85	PN1	T85A	MIC	Linezolid	No effect	/	/	/	/	/	27161641									
				Chloramphenicol																
				Cefuroxime																
				Oxacillin																
				Clindamycin																
				Tetracycline																
				Tigecycline																
				Levofloxacin																
				Novobiocin																
				Rifampicin																
				Clarithromycin								Increased MIC (400% activity)								
				Erythromycin								No effect								
				Azithromycin																
				Erythromycin																
				Q89								PN1: deen binding pocket	Q89C	MIC	Erythromycin	/	/	No effect	/	/

Strain	PN1, deep mining project	Strain	MIC	Antibiotic	WT	Mutant	WT Effect	Mutant Effect	Notes	Residue	Accession
D99	PN1	CysLess: D99C Cys was replaced by Ala	MIC	Novobiocin	/	/	No effect	/	well expressed; no differences between mutants	/	14576158
				Chloramphenicol	/	/	No effect	/			
				Tetracycline	/	/	No effect	/			
				Minocycline	/	/	No effect	/			
				Erythromycin	/	/	No effect	/			
				Nalidixic acid	/	/	No effect	/			
				Norfloracin	/	/	No effect	/			
				Enoxacin	/	/	No effect	/			
				Novobiocin	/	/	No effect	/			
				Acriflavine	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Crystal violet	/	/	No effect	/			
				Ethidium Bromide	/	/	No effect	/			
				Rhodamine 6G	/	/	No effect	/			
				TPP	/	/	No effect	/			
Benzalkonium	/	/	No effect	/							
SDS	/	/	No effect	/							
Deoxycholate	/	/	No effect	/							
A100	PN1	CysLess: A100C Cys was replaced by Ala	MIC	Chloramphenicol	/	/	No effect	/	well expressed; no differences between mutants	/	14576158
				Tetracycline	/	/	No effect	/			
				Minocycline	/	/	No effect	/			
				Erythromycin	/	/	No effect	/			
				Nalidixic acid	/	/	No effect	/			
				Norfloracin	/	/	No effect	/			
				Enoxacin	/	/	No effect	/			
				Novobiocin	/	/	No effect	/			
				Acriflavine	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Crystal violet	/	/	No effect	/			
				Ethidium Bromide	/	/	No effect	/			
				Rhodamine 6G	/	/	No effect	/			
				TPP	/	/	No effect	/			
				Benzalkonium	/	/	No effect	/			
SDS	/	/	No effect	/							
Deoxycholate	/	/	No effect	/							
		A100W	MIC on agar plates	Benzalkonium chloride	/	/	No effect	/	Comparable to WT	Residue was identified using PDB 3AOD	29317622
				Rhodamine 6G	/	/	No effect	/			
				Ethidium bromide	/	/	No effect	/			
				Acriflavine	/	/	No effect	/			
				Crystal violet	/	/	No effect	/			
				Minocycline	/	/	No effect	/			
				Cloxacillin	/	/	No effect	/			
				Dequalinium	/	/	No effect	/			
				Enoxacin	/	/	No effect	/			
				Norfloracin	/	/	No effect	/			
				Novobiocin	/	/	No effect	/			
				Erythromycin	/	/	No effect	/			
				Rifampicin	/	/	No effect	/			
				Novobiocin	/	/	No effect	/			
Tetracycline	/	/	No effect	/							
Norfloracin	/	/	No effect	/							
Erythromycin	/	/	No effect	/							
Taurocholic acid	/	/	No effect	/							
Ethidium	/	/	No effect	/							
Dequalinium	/	/	No effect	/							
Rhodamin-6-G	/	/	No effect	/							
Acriflavine	/	/	No effect	/							
SDS	/	/	No effect	/							
D101	PN1; central cavity	D101A	MIC	Novobiocin	/	/	No effect	/	comparable to WT	/	16166543
				Tetracycline	/	/	No effect	/			
				Norfloracin	/	/	No effect	/			
				Erythromycin	/	/	No effect	/			
				Ethidium	/	/	No effect	/			
				Dequalinium	/	/	No effect	/			
				Rhodamin-6-G	/	/	No effect	/			
	Acriflavine			/	/	No effect	/				
	SDS			/	/	No effect	/				
	Chloramphenicol			/	/	100% inhibition	Equal activity				
	Tetracycline			/	/	No effect	/				
	Minocycline			/	/	Intermediate effect (~ 25 % of WT)	4x activity of neg ctrl (ΔAcrB)				
	Erythromycin			/	/	100% inhibition	Equal activity				
	Nalidixic acid			/	/	No effect	/				
Norfloracin	/	/	No effect	/							
Enoxacin	/	/	No effect	/							
Novobiocin	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)							
Acriflavine	/	/	Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (ΔAcrB)							
Crystal violet	/	/	No effect	/							
Ethidium Bromide	/	/	Intermediate effect (~ 6 % of WT)	32x activity of neg ctrl (ΔAcrB)							
Rhodamine 6G	/	/	No effect	/							
TPP	/	/	No effect	/							
Benzalkonium	/	/	Intermediate effect (~ 25 % of WT)	4x activity of neg ctrl (ΔAcrB)							
SDS	/	/	No effect	/							
Deoxycholate	/	/	No effect	/							
				Chloramphenicol	/	/	No effect	/			
				Tetracycline	/	/	No effect	/			
				Minocycline	/	/	No effect	/			
				Erythromycin	/	/	No effect	/			
				Nalidixic acid	/	/	No effect	/			
				Norfloracin	/	/	No effect	/			
				Enoxacin	/	/	No effect	/			

I102C	PN1	CysLess: I102C Cys was replaced by Ala	MIC	Novobiocin	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)	well expressed; no differences between mutants	/	14576158
				Acriflavine			No effect	/			
A103	PN1	CysLess: A103C Cys was replaced by Ala	MIC	Crystal violet	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)	well expressed; no differences between mutants	/	14576158
				Ethidium Bromide			No effect	/			
Q104	PN1	CysLess: Q104C Cys was replaced by Ala	MIC	Rhodamine 6G	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)	well expressed; no differences between mutants	/	14576158
				TPP			No effect	/			
V105	PN1	CysLess: V105C Cys was replaced by Ala	MIC	Benzalkonium	/	/	100% inhibition	Equal activity	well expressed; no differences between mutants	/	14576158
				SDS			Intermediate effect (~ 6% of WT)	2x activity of neg ctrl (ΔAcrB)			
Q106	PN1	CysLess: Q106C Cys was replaced by Ala	MIC	Deoxycholate	/	/	100% inhibition	Equal activity	well expressed; no differences between mutants	/	14576158
				Chloramphenicol			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Tetracycline			No effect	/			
				Minocycline			Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (ΔAcrB)			
				Erythromycin			100% inhibition	Equal activity			
				Nalidixic acid			No effect	/			
				Norflloxacin			Intermediate effect (~ 6% of WT)	2x activity of neg ctrl (ΔAcrB)			
				Enoxacin			100% inhibition	Equal activity			
				Novobiocin			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Acriflavine			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Crystal violet			Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (ΔAcrB)			
				Ethidium Bromide			Intermediate effect (~ 6% of WT)	2x activity of neg ctrl (ΔAcrB)			
				Rhodamine 6G			100% inhibition	Equal activity			
				TPP			Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (ΔAcrB)			
				Benzalkonium			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				SDS			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Deoxycholate			Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (ΔAcrB)			
				Chloramphenicol			100% inhibition	Equal activity			
				Tetracycline			No effect	/			
				Minocycline			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Erythromycin			No effect	/			
				Nalidixic acid			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Norflloxacin			No effect	/			
				Enoxacin			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Novobiocin			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Acriflavine			100% inhibition	Equal activity			
				Crystal violet			No effect	/			
				Ethidium Bromide			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Rhodamine 6G			No effect	/			
				TPP			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Benzalkonium			No effect	/			
				SDS			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Deoxycholate			No effect	/			
				Chloramphenicol			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Tetracycline			No effect	/			
				Minocycline			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			
				Erythromycin			No effect	/			
				Nalidixic acid			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)			

V107	PN1	CysLess: V107C Cys was replaced by Ala	MIC	Norfloxacin	/	/		well expressed; no differences between mutants	/	14576158	
				Enoxacin			Intermediate effect (~12.5 % of WT)				4x activity of neg ctrl (ΔAcrB)
Q108	PN1	CysLess: Q108C Cys was replaced by Ala	MIC	Novobiocin	/	/		well expressed; no differences between mutants	/	14576158	
				Acriflavine			Intermediate effect (~12.5 % of WT)				4x activity of neg ctrl (ΔAcrB)
	PN1; central pore of periplasmic domain	N109A	MIC	Crystal violet	/	/		comparable to WT	Mut-Apoprotein (1T9T); Cocrystal Mut by Ciprofloxacin (1T9U), Rhodamine 6G (1T9V), Nafillin (1T9W), Ethidium (1T9X), Phe-Arg- β-naphthylamide (1T9Y)		
				Ethidium Bromide			Intermediate effect (~25 % of WT)				4x activity of neg ctrl (empty vector)
				Rhodamine 6G			No effect				/
				TPP			Intermediate effect (~20% of WT)				6x activity of neg ctrl (empty vector)
				Benzalkonium			No effect				/
				SDS			No effect				/
				Deoxycholate			No effect				/
				Novobiocin			No effect				/
				Tetracycline			No effect				/
				Norfloxacin			No effect				/
	N109: PN1_D566: between TM7 and PC1	N109A_D566A	MIC	Erythromycin	/	/		comparable to WT			
				Taurocholic acid			Intermediate effect (~12-25 % of WT)				8x activity of neg ctrl (empty vector)
				Ethidium			Intermediate effect (~25 % of WT)				4x activity of neg ctrl (empty vector)
				Dequalinium			Intermediate effect (~25 % of WT)				2x activity of neg ctrl (empty vector)
				Rhodamin-6-G			Intermediate effect (~12-25 % of WT)				4x activity of neg ctrl (empty vector)
				Acriflavine			No effect				/
				SDS			Intermediate effect (~12-25 % of WT)				64x activity of neg ctrl (empty vector)
				Novobiocin			Intermediate effect (~25 % of WT)				16x activity of neg ctrl (empty vector)
				Tetracycline			Intermediate effect (~25 % of WT)				8x activity of neg ctrl (empty vector)
				Norfloxacin			Intermediate effect (~20% of WT)				6x activity of neg ctrl (empty vector)
	N109: PN1_F664: PC1	N109A_F664A	MIC	Erythromycin	/	/		comparable to WT			
				Taurocholic acid			Intermediate effect (~12 % of WT)				8x activity of neg ctrl (empty vector)
				Ethidium			Intermediate effect (~25 % of WT)				2x activity of neg ctrl (empty vector)
				Dequalinium			Intermediate effect (~25 % of WT)				4x activity of neg ctrl (empty vector)
				Rhodamin-6-G			No effect				/
				Acriflavine			100% inhibition				Equal activity
				SDS			Intermediate effect (~3 % of WT)				8x activity of neg ctrl (empty vector)
				Novobiocin			No effect				/
				Tetracycline			Intermediate effect (~25% of WT)				2x activity of neg ctrl (empty vector)
				Norfloxacin			Intermediate effect (~6 % of WT)				2x activity of neg ctrl (empty vector)
	N109: PN1_F666: PC1	N109A_F666A	MIC	Erythromycin	/	/		comparable to WT			
				Taurocholic acid			Intermediate effect (~25 % of WT)				4x activity of neg ctrl (empty vector)
				Ethidium			No effect				/
				Dequalinium			100% inhibition				Equal activity
				Rhodamin-6-G			Intermediate effect (~25 % of WT)				4x activity of neg ctrl (empty vector)
				Acriflavine			No effect				/
				SDS			Intermediate effect (~25 % of WT)				8x activity of neg ctrl (empty vector)
				Novobiocin			No effect				/
				Tetracycline			Intermediate effect (~25% of WT)				2x activity of neg ctrl (empty vector)
				Norfloxacin			Intermediate effect (~6 % of WT)				2x activity of neg ctrl (empty vector)
	N109: PN1_F673: PC1	N109A_E673A	MIC	Erythromycin	/	/		comparable to WT		16166543	
				Taurocholic acid			Intermediate effect (~12 % of WT)				2x activity of neg ctrl (empty vector)
				Ethidium			Intermediate effect (~12-25 % of WT)				2x activity of neg ctrl (empty vector)
				Dequalinium			Intermediate effect (~12-25 % of WT)				2x activity of neg ctrl (empty vector)
				Rhodamin-6-G			Intermediate effect (<1 % of WT)				2x activity of neg ctrl (empty vector)
				Acriflavine			Intermediate effect (~6-12 % of WT)				4x activity of neg ctrl (empty vector)
				SDS			Intermediate effect (~25% of WT)				4x activity of neg ctrl (empty vector)
				Novobiocin			No effect				/
				Tetracycline			Intermediate effect (~25% of WT)				4x activity of neg ctrl (empty vector)
				Norfloxacin			Intermediate effect (~6 % of WT)				4x activity of neg ctrl (empty vector)

N109	N109: PN1_R717: PC2	N109A_R7176A	MIC	Acriflavine	/	/	Intermediate effect (~6% of WT)	8x activity of neg ctrl (empty vector)	comparable to WT	/		
				SDS			100% inhibition	Equal activity				
				Novobiocin			Intermediate effect (~12% of WT)	8x activity of neg ctrl (empty vector)				
				Tetracycline			Intermediate effect (~12% of WT)	2x activity of neg ctrl (empty vector)				
				Norfloxacin			No effect	/				
				Erythromycin								
				Taurocholic acid								
				Ethidium								
				Dequalinium								
				Rhodamin-6-G								
				Acriflavine								
				SDS							Intermediate effect (~12-25% of WT)	8x activity of neg ctrl (empty vector)
				Novobiocin							Intermediate effect (~25% of WT)	8x activity of neg ctrl (empty vector)
				Novobiocin							No effect	/
				Tetracycline			Intermediate effect (~25% of WT)	4x activity of neg ctrl (empty vector)				
Norfloxacin	No effect	/										
Erythromycin	Intermediate effect (~25% of WT)	8x activity of neg ctrl (empty vector)										
Taurocholic acid	No effect	/										
Ethidium												
Dequalinium												
Rhodamin-6-G												
Acriflavine												
SDS			Intermediate effect (~10% of WT)	3x activity of neg ctrl (empty vector)								
Novobiocin			No effect	/								
Tetracycline			Intermediate effect (~25% of WT)	4x activity of neg ctrl (empty vector)								
Norfloxacin			No effect	/								
Erythromycin												
Taurocholic acid												
Ethidium												
Dequalinium												
Rhodamin-6-G												
Acriflavine												
SDS	Intermediate effect (~10% of WT)	3x activity of neg ctrl (empty vector)										
Novobiocin	Intermediate effect (~12% of WT)	8x activity of neg ctrl (empty vector)										
Tetracycline	100% inhibition	Equal activity										
Norfloxacin	No effect	/										
Erythromycin												
Taurocholic acid												
Ethidium												
Dequalinium												
Rhodamin-6-G												
Acriflavine												
SDS			Intermediate effect (~12-25% of WT)	8x activity of neg ctrl (empty vector)								
Novobiocin			100% inhibition	Equal activity								
Tetracycline			Intermediate effect (~25% of WT)	2x activity of neg ctrl (ΔAcrB)								
Minocycline	100% inhibition	Equal activity										
Erythromycin	No effect	/										
Nalidixic acid	Intermediate effect (~25% of WT)	4x activity of neg ctrl (ΔAcrB)										
Norfloxacin	100% inhibition	Equal activity										
Enoxacin	No effect	/										
Novobiocin												
Acriflavine												
Crystal violet			Intermediate effect (~12.5% of WT)	4x activity of neg ctrl (ΔAcrB)								
Ethidium Bromide			Intermediate effect (~6% of WT)	2x activity of neg ctrl (ΔAcrB)								
Rhodamine 6G			No effect	/								
TPP			Intermediate effect (~6% of WT)	16x activity of neg ctrl (ΔAcrB)								
Benzalkonium			No effect	/								
SDS			Intermediate effect (~25% of WT)	4x activity of neg ctrl (ΔAcrB)								
Deoxycholate			No effect	/								
Chloramphenicol	No effect	No effect										
Tetracycline												
Minocycline												
Erythromycin												
Nalidixic acid												
Norfloxacin												
Enoxacin												
Novobiocin												
Acriflavine												
Crystal violet			Intermediate effect (~12.5% of WT)	4x activity of neg ctrl (ΔAcrB)								
Ethidium Bromide	No effect	No effect										
Rhodamine 6G												
TPP												
Benzalkonium												
SDS												
Deoxycholate												
Chloramphenicol												
K110			PN1	CysLess: K110C Cys was replaced by Ala	MIC	/	/	well expressed; no differences between mutants	/	14576158		

L111	PN1	CysLess: L111C Cys was replaced by Ala	MIC	Tetracycline	/	/	No effect	No effect	well expressed; no differences between mutants	/	14576158									
				Minocycline			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)												
				Erythromycin			No effect	No effect												
				Nalidixic acid																
				Norfloracin																
				Enoxacin																
				Novobiocin																
				Acriflavine																
				Crystal violet																
				Ethidium Bromide																
				Rhodamine 6G																
				TPP																
				Benzalkonium																
				SDS																
				Deoxycholate																
Q112	PN1	Q112H_G141D_N282Y	MIC	Levofloxacin	No effect	/	/	/	/	/	25182653									
				Chloramphenicol																
				Oxacillin	Intermediate effect	activity of neg ctrl (ΔAcrB)														
				Linezolid	Intermediate effect	activity of neg ctrl (ΔAcrB)														
				Minocycline																
				Novobiocin	No effect	/														
	MIC + inhibitor (NMP)	Levofloxacin	No effect	/																
		Chloramphenicol																		
		Oxacillin	Intermediate effect	activity of neg ctrl (ΔAcrB)																
		Linezolid	Intermediate effect	activity of neg ctrl (ΔAcrB)																
		Minocycline																		
		Novobiocin	No effect	/																
Q112	PN1	CysLess: Q112C Cys was replaced by Ala	MIC	Clarithromycin	/	/	100% inhibition	Equal activity	well expressed; no differences between mutants	/	14576158									
				Rifampicin			Intermediate effect (~ 25 % of WT)	4x activity of neg ctrl (ΔAcrB)												
				Chloramphenicol			100% inhibition	Equal activity												
				Tetracycline			Intermediate effect (~ 25 % of WT)	4x activity of neg ctrl (ΔAcrB)												
				Minocycline			Intermediate effect (~ 6% of WT)	2x activity of neg ctrl (ΔAcrB)												
				Erythromycin			100% inhibition	Equal activity												
				Nalidixic acid			Intermediate effect (~ 12.5% of WT)	4x activity of neg ctrl (ΔAcrB)												
				Norfloracin			Intermediate effect (~ 3% of WT)	4x activity of neg ctrl (ΔAcrB)												
				Enoxacin			Intermediate effect (~ 6% of WT)	2x activity of neg ctrl (ΔAcrB)												
				Novobiocin			100% inhibition	Equal activity												
				Acriflavine			Intermediate effect (~ 25 % of WT)	2x activity of neg ctrl (ΔAcrB)												
				Crystal violet			No effect	/												
				TPP																
				Benzalkonium																
				SDS																
				Deoxycholate																
				Chloramphenicol																
				L113			PN1	CysLess: L113C Cys was replaced by Ala				MIC	Tetracycline	/	/	No effect	No effect	well expressed; no differences between mutants	/	14576158
Minocycline	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)																		
Erythromycin																				
Nalidixic acid																				
Norfloracin																				
Enoxacin																				
Novobiocin																				
Acriflavine																				
Crystal violet																				
Ethidium Bromide																				
Rhodamine 6G																				
TPP																				
Benzalkonium																				
SDS																				
Deoxycholate																				
Chloramphenicol																				
A114	PN1	CysLess: A114C Cys was replaced by Ala	MIC		Tetracycline	/			/	No effect	No effect		well expressed; no differences between mutants			/	14576158			
					Minocycline					Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)									
				Erythromycin																
				Nalidixic acid																
				Norfloracin																
				Enoxacin																
				Novobiocin																
				Acriflavine																
				Crystal violet																
				Ethidium Bromide																
				Rhodamine 6G																
				TPP																
				Benzalkonium																
				SDS																
				Deoxycholate																
				Chloramphenicol																

M115	PN1	CysLess: M115C Cys was replaced by Ala	MIC	SDS	/	/	No effect	No effect	well expressed; no differences between mutants	/	14576158
				Deoxycholate							
				Chloramphenicol							
				Tetracycline							
				Minocycline							
				Erythromycin							
				Nalidixic acid							
				Norfloxacine							
				Enoxacin							
				Novobiocin							
				Acriflavine							
				Crystal violet							
				Ethidium Bromide							
				Rhodamine 6G							
P116	PN1	CysLess: P116C Cys was replaced by Ala	MIC	TPP	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (ΔAcrB)	well expressed; no differences between mutants	/	14576158
				Benzalkonium							
				SDS							
				Deoxycholate							
				Chloramphenicol							
				Tetracycline							
				Minocycline							
				Erythromycin							
				Nalidixic acid							
				Norfloxacine							
				Enoxacin							
				Novobiocin							
				Acriflavine							
				Crystal violet							
L117	PN1	CysLess: L117C Cys was replaced by Ala	MIC	Ethidium Bromide	/	/	No effect	/	well expressed; no differences between mutants	/	14576158
				Rhodamine 6G							
				TPP							
				Benzalkonium							
				SDS							
				Deoxycholate							
				Chloramphenicol							
				Tetracycline							
				Minocycline							
				Erythromycin							
				Nalidixic acid							
				Norfloxacine							
				Enoxacin							
				Novobiocin							
Acriflavine											
L118	PN1	CysLess: L118C Cys was replaced by Ala	MIC	Crystal violet	/	/	No effect	No effect	well expressed; no differences between mutants	/	14576158
				Ethidium Bromide							
				Rhodamine 6G							
				TPP							
				Benzalkonium							
				SDS							
				Deoxycholate							
				Chloramphenicol							
				Tetracycline							
				Minocycline							
				Erythromycin							
				Nalidixic acid							
				Norfloxacine							
				Enoxacin							
Novobiocin											
P119	PN1	CysLess: P119C Cys was replaced by Ala	MIC	Acriflavine	/	/	No effect	No effect	well expressed; no differences between mutants	/	14576158
				Crystal violet							
				Ethidium Bromide							

				Rhodamine 6G			No effect	No effect				
				TPP								
				Benzalkonium								
				SDS								
				Deoxycholate								
V127	PN1	V127A	MIC	Erythromycin	/	/	Intermediate effect	16x activity of neg ctrl (Δ AcxAB)	/	/	26240069	
				Novobiocin								
				Minocycline			No effect	/				
				Nalidixic acid								
		V127G	MIC	Erythromycin	/	/	No effect	/	/	/		
				Novobiocin								
				Minocycline								
				Nalidixic acid								
E130	E130: PN1_E269: DN_M355: TM2_V448: TM5_T495: between TM6 and TM7	30G_E269G_M355L_V448A_T49	Growth assay	Isobutanol	/	/	Enhanced growth	Enhanced growth	Comparable to WT	/	23991711	
				n-Pentanol				No neg ctrl; only comp to WT-AcrB				
				n-Hexanol								
				n-Heptanol								
				n-Octanol								
				Ampicillin			No effect	/				
				Chloramphenicol								
				Tetraphenylphosphonium								
S132	PN1	CysLess: S132C Cys was replaced by Ala	MIC	Rhodamine 6G	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659	
				Berberine								
				Erythromycin								
				Oxacillin								
	S132: PN1_A294: PN2; interface	CysLess: S132C_A294C Cys was replaced by Ala		Tetraphenylphosphonium	/	/	Intermediate effect (~12% of WT activity)	16x activity of neg ctrl (empty vector)	Comparable to other mutants and ctrl	/		
				Rhodamine 6G			Intermediate effect (~12-25% of WT activity)	8x activity of neg ctrl (empty vector)				
				Berberine			Intermediate effect (~25% of WT activity)	4x activity of neg ctrl (empty vector)				
				Erythromycin			No effect	/				
				Oxacillin			Intermediate effect (~6-12% of WT activity)	4x activity of neg ctrl (empty vector)				
S133	Between PN1 and PN2	CysLess: S133C Cys was replaced by Ala	MIC	Novobion	/	/	No effect	/	/	/	11754746	
		S134C	MIC	Erythromycin	/	/	No effect	/	/	/	24551234	
				Novobion								
S134	Between PN1 and PN2; deep binding pocket		Growth inhibition	Erythromycin	/		Decreased growth compared to WT	Slightly higher growth comp to neg ctrl (empty vector)	Comparable to WT	/	22121023	
		S134A_S135A	Accumulation Assay of Doxorubicin	None; only Doxorubicin	/		No effect	/	Comparable to WT	/		
				Erythromycin	/		Intermediate effect: no inhibition of doxo efflux	Difference comp to neg ctrl (empty vector); in ctrl Doxo is highly accumulated; while it is not in mutant	Comparable to WT	/		
		F136A		No effect on activity; but was not further tested here; they referred to paper with PMID 18849422 (see below)						Comparable to WT	/	26240069
				Oxacillin	Intermediate effect (~25% of WT)	12x activity of neg ctrl (inactivation mutant)						
		F136A	MIC	Novobiocin	Intermediate effect (~12.5% of WT)	16x activity of neg ctrl (inactivation mutant)	/	/	Comparable to WT	/	18849422	
				Clarithromycin								
				Erythromycin								
			Efflux assay	Ethidium bromide	No effect	/	/	/				
				PAbetaN								
F136	PN2; hydrophobic pocket		MIC	1,2'-dinaphthylamine	No effect	/						
		F136A	Efflux assay (efflux half times)	2'-dinaphthylamine 0.25 μ M	Intermediate effect	neg ctrl for efflux half t	/	/	Comparable to WT	/	21698261	
				1,2'-dinaphthylamine 1 μ M								
				1,2'-dinaphthylamine 4 μ M								
				1,2'-dinaphthylamine 16 μ M								
				1,2'-dinaphthylamine 64 μ M								
		F136A	Efflux assay	1,2'-dinaphthylamine 4 μ M	Intermediate effect	Lower decrease comp	/	/				
		F136A	Efflux assay	Nitrocefin	/	/	No effect on K_{M} , V_{max} , C_p	/	/	/	27789287	
				Ala-Naph			Cp increased; no effect on K_{M} , V_{max}	no neg ctrl				
		F136C	MIC	Erythromycin	/	/	No effect	/	/	/	24551234	
				Novobion								
V139	PN2	V139F	cefin efflux in presence of other il	Nitrocefin; no additive	K_{M} : no effect V_{max} : no effect	/	/	/	/	/	24205856	
				Nitrocefin; additive Benzen	K_{M} : intermediate effect	with dead mutant or						
					V_{max} : no effect	/						
				rocefin; additive Cyclohexa	K_{M} : no effect	/						
					V_{max} : intermediate effect	with dead mutant or						
				rocefin; additive Cyclohexar	K_{M} : no effect	/						
					V_{max} : no effect	/						
				Levofloxacin	No effect	/	/	/	/	/		
				Chloramphenicol								
				Oxacillin								
				Linezolid								
				Minocycline								
				Novobiocin								
				Clarithromycin								
				Rifampicin								
		G141D		Levofloxacin	No effect	/						
				Chloramphenicol								

G141	PN2	G141D N282Y	MIC + inhibitor (NMP)	Oxacillin	/	/	/	/	/	25182653		
				Linezolid	/	/	/	/	/			
				Minocycline	Not determined	/	/	/	/			
				Novobiocin	/	/	/	/				
				Clarithromycin	No effect	/	/	/				
				Rifampicin	/	/	/	/				
			MIC	Levofloxacin	/	/	/	/				
				Chloramphenicol	/	/	/	/				
				Oxacillin	/	/	/	/				
				Linezolid	No effect	/	/	/				
				Minocycline	/	/	/	/				
				Novobiocin	/	/	/	/				
			MIC + inhibitor (NMP)	Clarithromycin	/	/	/	/				
				Rifampicin	/	/	/	/				
Levofloxacin	No effect	/		/	/							
Chloramphenicol	/	/		/	/							
Oxacillin	/	/		/	/							
Linezolid	Intermediate effect (~25% of WT)	activity of neg ctrl (Δ AcB)		/	/							
	Minocycline	/	/	/	/							
	Novobiocin	No effect	/	/	/							
	Clarithromycin	/	/	/	/							
	Rifampicin	/	/	/	/							
N144	D144: PN2_I335: TM2_S1043: C-Terminus	N144S_I335T_S1043R	Growth assay	<i>n</i> -Butanol	/	/	Enhanced growth	Enhanced growth	Comparable to WT	/	23991711	
D153	PN2	D153E	MIC	Erythromycin	/	/	No effect	/	/	/	26240069	
				Novobiocin	/	/	/	/	/			
				Minocycline	/	/	/	/	/			
				Nalidixic acid	/	/	/	/	/			
D174	D174: PN2_K522: between TM6 and TM7_S880: TM8	D174N_K522G_S880P	Growth assay	<i>n</i> -Butanol	/	/	Enhanced growth	Enhanced growth	Comparable to WT	/	23991711	
Q176	PN2	Q176A	Efflux assay	Nitrocefin	/	/	No effect on K_{M0} , V_{max} , C_p	/	/	/	27789287	
				Ala-Naph	/	/	No effect on K_{M0} , V_{max} , C_p	/	/	/		
F178	PN2; distal binding pocket; hydrophobic trap	F178W	Growth in presence of ABI-PP	Erythromycin	/	/	Difference to WT: No growth inhibition of mutant (concl: ABI-PP cannot bind mutant)	Higher growth than neg ctrl (Δ AcB)	Comparable to WT	Yes	23812586	
				Accumulation Assay w/o ABI-PP	Doxorubicin	/	/	No effect (No accumulation of Doxorubicin)				/
			Accumulation Assay in presence of ABI-PP	Doxorubicin	/	/	Small difference to WT: Doxorubicin is not accumulated and can be extruded by the cell with and w/o ABI-PP (concl: ABI-PP cannot bind mutant)	Difference to neg ctrl I (Δ AcB): Doxo is accumulated in neg ctrl, but not in mutant				
			MIC	Oxacillin	/	/	No effect	/				
				Erythromycin	/	/	/	/				
				Doxorubicin	/	/	/	/				
				Minocycline	/	/	/	/				
				Rhodamine 6G	/	/	/	/				
				Ethidium bromide	/	/	/	/				
		F178A	MIC	Oxacillin	Intermediate effect (~12.5% of WT)	64x activity of neg ctrl (inactivation mutant)	/	/	/	/	/	18849422
				Novobiocin	Intermediate effect (~3,1% of WT)	4x activity of neg ctrl (inactivation mutant)						
				Clarithromycin	Intermediate effect (~12.5% of WT)	16x activity of neg ctrl (inactivation mutant)						
				Erythromycin	Intermediate effect (~25% of WT)	32x activity of neg ctrl (inactivation mutant)						
				Azithromycin	/	/						
				Linezolid	Intermediate effect (~12.5% of WT)	4x activity of neg ctrl (inactivation mutant)						
				Hoechst 33342	Intermediate effect (~25% of WT)	2x activity of neg ctrl (inactivation mutant)						
		Efflux assay	Ethidium bromide	No effect	/	/						
			PAbetaN	Decreased efflux	Lower efflux comp to neg ctrl (inactivation mutant)	/						
		F178A	Efflux assay (efflux half times)	1,2'-dinaphthylamine	No effect	/	/	/	/	/	/	21698261
2'-dinaphthylamine 0.25 μ M	No effect			/								
1,2'-dinaphthylamine 1 μ M	Intermediate effect			neg ctrl for efflux half times								
1,2'-dinaphthylamine 4 μ M	/			/								
1,2'-dinaphthylamine 16 μ M	No effect			/								
Efflux assay	1,2'-dinaphthylamine 4 μ M	Intermediate effect	Lower decrease comp to neg ctrl (inactivation mutant)	/								
F178A	Efflux assay	Nitrocefin	/	/	Cp increased; no effect on K_{M0} , V_{max}	No neg ctrl	/	/	/	27789287		
		Ala-Naph	/	/								

M184	M184: DN_V771: DC	CysLess: M184C_V771C Cys was replaced by Ala	MIC	Erythromycin Novobiocin	/	/	No effect	/	Comparable to WT	/	21529338
W187	DN	W187A	MIC on agar plates	Acriflavine	/	/	No effect	/	Comparable to WT	/	24007302
				Erythromycin							
				Novobiocin							
				Rhodamine 6G							
N189	DN	N189H	Efflux rate: Hydrocarbon release rate constant/ min ⁻¹	Tetraphenylphosphonium	/	/	Intermediate effect (~25% of WT)	32x activity of neg ctrl (empty vector)	Comparable to WT	/	23693002
				<i>n</i> -octane			No effect	/			
				α -pinene			Increased efflux	/			
				<i>n</i> -octane			Increased efflux	/			
T199	T199: DN_T749: DC	CysLess: T199C_T749C Cys was replaced by Ala	MIC	Erythromycin	/	/	No effect	/	Comparable to WT	/	21529338
				Novobiocin							
N211	DN; protruding loop	N211A	MIC	SDS	/	/	No effect	/	Comparable to WT	Only structure model	22877148
				Erythromycin							
				Rhodamine 6G							
				Novobiocin							
				Acriflavine							
Q213	DN; protruding loop	Q213A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	Only structure model	22877148
				SDS							
				Erythromycin							
				Rhodamine 6G							
				Novobiocin							
V214	DN; protruding loop	V214A	MIC	Novobiocin	/	/	No effect	/	Less expr comp to WT	Only structure model	22877148
				Acriflavine							
				Tetraphenylphosphonium							
				SDS							
				Erythromycin							
A216	A216: DN_I234: DN	CysLess: A216C_I234C Cys was replaced by Ala	MIC	Erythromycin	/	/	No effect	/	/	Only structure model	22163011
				Novobiocin							
				Rhodamine 6G							
				Tetraphenylphosphonium							
G217	DN; protruding loop	G217A	MIC	Tetracycline	/	/	Intermediate effect (~ 25 % of WT)	20x activity of neg ctrl (empty vector)	Less expr comp to WT	Only structure model	22877148
				SDS			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (empty vector)			
				Erythromycin			Intermediate effect (~ 25 % of WT)	16x activity of neg ctrl (empty vector)			
				Rhodamine 6G			Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (empty vector)			
				Novobiocin			No effect	/			
				Acriflavine			Intermediate effect (~ 12.5 % of WT)	16x activity of neg ctrl (empty vector)			
Q218	DN; protruding loop	Q218A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	Only structure model	22877148
				SDS							
				Erythromycin							
				Rhodamine 6G							
				Novobiocin							
L219	DN; protruding loop	L219A	MIC	Novobiocin	/	/	Intermediate effect (~ 6-12.5 % of WT)	5x activity of neg ctrl (empty vector)	Comparable to WT	Only structure model	22877148
				Acriflavine			No effect	/			
				Tetraphenylphosphonium			Intermediate effect (~ 6-12.5 % of WT)	4x activity of neg ctrl (empty vector)			
				SDS			Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (empty vector)			
				Erythromycin			No effect	/			
				Rhodamine 6G			Intermediate effect (~ 1.5 % of WT)	2x activity of neg ctrl (empty vector)			
G220	DN; protruding loop	G220A	MIC	Tetraphenylphosphonium	/	/	Intermediate effect (~ 6 % of WT)	80x activity of neg ctrl (empty vector)	Comparable to WT	Only structure model	22877148
				SDS			Intermediate effect (~ 25 % of WT)	2x activity of neg ctrl (empty vector)			
				Erythromycin			Intermediate effect (~ 12.5 % of WT)	16x activity of neg ctrl (empty vector)			
				Rhodamine 6G			No effect	/			
				Novobiocin			Intermediate effect (~ 3 % of WT)	4x activity of neg ctrl (empty vector)			
G221	DN; protruding loop	G221A	MIC	Novobiocin	/	/	No effect	/	Comparable to WT	Only structure model	22877148
				Acriflavine							
				Rhodamine 6G							
				Erythromycin							
T222	DN; protruding loop	T222A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Less expr comp to WT	Only structure model	22877148
				SDS							
				Erythromycin							
				Rhodamine 6G							
				Novobiocin							
P223G	DN; protruding loop	P223G	MIC	Tetraphenylphosphonium	/	/	Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (empty vector)	/	/	/
				SDS			Intermediate effect (~ 3-6 % of WT)	2x activity of neg ctrl (empty vector)			
				Erythromycin			Intermediate effect (~ 3-6 % of WT)	4x activity of neg ctrl (empty vector)			
				Erythromycin + DTT			Intermediate effect (~ 3 % of WT)	4x activity of neg ctrl (empty vector)			
				Novobiocin			100% inhibition	Equal activity			
				Rhodamine 6G							

P223	DN; protruding loop (for trimer stability)	P223A	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline	/	/	Intermediate effect (~ 12.5 % of WT)	4x activity of neg ctrl (empty vector)	Comparable to WT	Only structure model	22163011				
		P223V	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline			Intermediate effect (~ 12.5-25 % of WT)	8x activity of neg ctrl (empty vector)							
						Intermediate effect (~ 6 % of WT)	8x activity of neg ctrl (empty vector)							
		P223Y	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline			No effect	/							
						Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (empty vector)							
		P223N	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline			Intermediate effect (~ 6-12.5 % of WT)	4x activity of neg ctrl (empty vector)							
	Intermediate effect (~ 3 % of WT)			4x activity of neg ctrl (empty vector)										
	CysLess: P223G_A216C_I234C Cys was replaced by Ala	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline	No effect	/										
	CysLess: P223G_V225C Cys was replaced by Ala	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline	Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (empty vector)										
	CysLess: P223G_V225C_A777C Cys was replaced by Ala	Erythromycin Novobiocin Rhodamine 6G Tetraphenylphosphonium Tetracycline	Intermediate effect (~ 6-12.5 % of WT)	4x activity of neg ctrl (empty vector)										
	P223	DN; protruding loop	P223G	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium +D	/	/	Intermediate effect (~ 6 % of WT)				2x activity of neg ctrl (empty vector)	Comparable to WT	Only structure model	25657644
			P223: DN; protruding loop_T199: DN; docking domain	P223G_T199M			Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium				Intermediate effect (~ 12.5 % of WT)			
Intermediate effect (~ 25 % of WT)								4x activity of neg ctrl (empty vector)						
P223: DN; protruding loop_A209: DN; docking domain			P223G_A209V	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium			No effect	/						
							Intermediate effect (~ 3-6 % of WT)	2x activity of neg ctrl (empty vector)						
P223: DN; protruding loop_D256: DN; docking domain			P223G_D256N	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium			Intermediate effect (~ 6 % of WT)	2x activity of neg ctrl (empty vector)						
							Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (empty vector)						
P223: DN; protruding loop_G257: DN; docking domain			P223G_G257V	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium			No effect	/						
							Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (empty vector)						
P223: DN; protruding loop_M662: DN; porter domain			P223G_M662I	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium			No effect	/						
							Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (empty vector)						
P223: DN; protruding loop_Q737: DN; docking domain			P223G_Q737I	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium			No effect	/						
	Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (empty vector)												
P223: DN; protruding loop_Q788: DN; docking domain	P223G_D788K	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium	No effect	/										
			Intermediate effect (~ 25 % of WT)	8x activity of neg ctrl (empty vector)										
P223: DN; protruding loop_P800: DN; docking domain	P223G_P800S	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium	No effect	/										
			Intermediate effect (~ 12 % of WT)	8x activity of neg ctrl (empty vector)										
P223: DN; protruding loop_E810: DN; docking domain	P223G_E810K	Erythromycin Novobiocin Tetracycline Tetraphenylphosphonium	No effect	/										
			Intermediate effect (~ 12 % of WT)	4x activity of neg ctrl (empty vector)										
			SDS			No effect	/							
			Erythromycin			Intermediate effect (~ 12 % of WT)	4x activity of neg ctrl (empty vector)							

	DN; protruding loop	P223A	MIC	Rhodamine 6G Novobiocin Acriflavine Tetraphenylphosphonium	/	/	Intermediate effect (~ 25 % of WT) Intermediate effect (~ 12 % of WT) No effect Intermediate effect (~ 6 % of WT)	16x activity of neg ctrl (empty vector) 4x activity of neg ctrl (empty vector) / 5x activity of neg ctrl (empty vector)	Less expr comp to WT	Only structure model	22877148		
P224	DN ; protruding loop	P224T	MIC	Linezolid	No effect	/							
				Chloramphenicol	Intermediate effect (~ 25 % of WT)	activity of neg ctrl (ΔA							
				Cefuroxime	No effect	/	/	/	/	/	/	/	/
				Oxacillin									
				Clindamycin									
				Tetracycline									
				Tigecycline									
				Levofloxacin									
				Novobiocin									
				Rifampicin									
				Clarithromycin	Increased MIC (400% activity)	/							
				Erythromycin	No effect	/							
Azithromycin	No effect	/											
P224L_F316S	MIC	Linezolid	No effect	/									
		Chloramphenicol	Intermediate effect (~ 25 % of WT)	activity of neg ctrl (ΔA									
		Cefuroxime	No effect	/	/	/	/	/	/	/	/		
		Oxacillin											
		Clindamycin											
		Tetracycline											
		Tigecycline											
		Levofloxacin											
		Novobiocin											
		Rifampicin											
		Clarithromycin	Increased MIC (400% activity)	/									
		Erythromycin	No effect	/									
Azithromycin	No effect	/											
P224G	MIC	Erythromycin											
		Novobiocin											
		Rhodamine 6G	/	/	No effect	/	/	/	Only structure model	22163011			
		Tetraphenylphosphonium											
P224A	MIC	Tetracycline											
		SDS											
		Erythromycin											
		Rhodamine 6G	/	/	No effect	/	/	/	Comparable to WT	Only structure model	22877148		
V225	DN; protruding loop	CysLess: V225C Cys was replaced by Ala	MIC	Rhodamine 6G	/	/	No effect	/		Comparable to other mutants and ctrl	/	18223659	
				Berberine									
				Erythromycin									
				Oxacillin									
	V225: DN_A777:PC2; interface loop-DC	CysLess: V225C_A777C Cys was replaced by Ala	MIC	Tetraphenylphosphonium									
				Rhodamine 6G	/	/	No effect	/	/				
				Berberine									
				Oxacillin									
	V225: DN_A777:PC2; interface loop-DC	CysLess: V225C_A777C Cys was replaced by Ala	MIC on agar plates	Acriflavine									
				Erythromycin	/	/	No effect	/	/	Comparable to WT	/	24007302	
				Novobiocin									
				Rhodamine 6G	/	/	No effect	/	/				
DN; protruding loop	V225C	MIC	Tetraphenylphosphonium										
			Erythromycin	/	/	No effect	/	/	/	Only structure model	22163011		
			Novobiocin										
			Rhodamine 6G	/	/	No effect	/	/					
V225: DN; protruding loop_A777:DC	CysLess: V225C_A777C Cys was replaced by Ala	MIC	Tetraphenylphosphonium										
			Erythromycin	/	/	No effect	/	/					
			Novobiocin										
			Rhodamine 6G	/	/	No effect	/	/					
DN; protruding loop	V225A	MIC	SDS										
			Erythromycin										
			Rhodamine 6G	/	/	No effect	/	/	/	Comparable to WT	Only structure model	22877148	
			Novobiocin										
DN; protruding loop	CysLess: V225C Cys was replaced by Ala	MIC	Acriflavine										
			Erythromycin	/	/	No effect	/	/	Lower than WT	/	22766312		
			Novobiocin										
			Rhodamine 6G	/	/	No effect	/	/					
K226	DN; protruding loop	K226A	MIC	Tetraphenylphosphonium									
				SDS									
				Erythromycin	/	/	No effect	/	/	/	Comparable to	Only structure model	22877148

G227	DN; protruding loop	G227A	MIC	Novobiocin	/	/	No effect	/	WT	Only structure model	22877148	
				Acriflavine								
				Tetraphenylphosphonium								
				SDS								
Q228	DN; protruding loop	Q228A	MIC	Erythromycin	/	/	No effect	/	Comparable to WT	Only structure model	22877148	
				Rhodamine 6G								
				Novobiocin								
				Acriflavine								
Q229	DN	CysLess: Q229C Cys was replaced by Ala	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659	
				Rhodamine 6G								
				Berberine								
				Erythromycin								
	Q229: DN_T583: PC1; interface loop PC1	CysLess: Q229C_T583C Cys was replaced by Ala	MIC	MIC	Oxacillin	/	/	Intermediate effect (~12% of WT)	16x activity of neg ctrl (empty vector)	Comparable to other mutants and ctrl	/	18223659
					Tetraphenylphosphonium							
					Rhodamine 6G			Intermediate effect (~6-12% of WT)				
					Berberine			Intermediate effect (~25% of WT)	4x activity of neg ctrl (empty vector)			
	Q229: DN_R586: PC1; interface loop PC1	CysLess: Q229C_R586C Cys was replaced by Ala	MIC	MIC	Erythromycin	/	/	Intermediate effect (~12% of WT)	4x activity of neg ctrl (empty vector)	Comparable to other mutants and ctrl	/	18223659
					Oxacillin							
					Tetraphenylphosphonium			No effect	/			
					Rhodamine 6G			Intermediate effect (~25% of WT)	16x activity of neg ctrl (empty vector)			
Q229A	DN; protruding loop	Q229A	MIC	Erythromycin	/	/	No effect	/	Comparable to WT	Only structure model	22877148	
				Rhodamine 6G								
				Novobiocin								
				Acriflavine								
L230	DN; protruding loop	L230A	MIC	Tetraphenylphosphonium	/	/	Intermediate effect (~12% of WT)	8x activity of neg ctrl (empty vector)	Comparable to WT	Only structure model	22877148	
				SDS			Intermediate effect (~6% of WT)	2x activity of neg ctrl (empty vector)				
				Erythromycin			Intermediate effect (~6% of WT)	4x activity of neg ctrl (empty vector)				
				Rhodamine 6G			Intermediate effect (~6% of WT)	4x activity of neg ctrl (empty vector)				
				Novobiocin			Intermediate effect (~25% of WT)	2x activity of neg ctrl (empty vector)				
				Acriflavine			Intermediate effect (~1.5% of WT)					
N231	DN; protruding loop	N231A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	Only structure model	22877148	
				SDS								
				Erythromycin								
				Rhodamine 6G								
S233	TM2	CysLess: S233C Cys was replaced by Ala	MIC	Erythromycin	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659	
				Rhodamine 6G								
	S233: TM2_Q726:DC; interface loop-DC	CysLess: S233C_Q726C Cys was replaced by Ala	MIC	MIC	Berberine	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659
					Erythromycin							
					Oxacillin							
					SDS							
S233A	DN; protruding loop	S233A	MIC	Erythromycin	/	/	No effect	/	Comparable to WT	Only structure model	22877148	
				Rhodamine 6G								
				Novobiocin								
				Acriflavine								
I234	DN; protruding loop	I234A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	Only structure model	22877148	
				SDS								
				Erythromycin								
				Rhodamine 6G								
I235	DN	CysLess: I235C Cys was replaced by Ala	MIC	Erythromycin	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659	
				Rhodamine 6G								
				Berberine								
				Oxacillin								
	I235: DN_K728:DC; interface loop-DC	CysLess: I235C_K728C Cys was replaced by Ala	MIC	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to other mutants and ctrl	/	18223659
					Rhodamine 6G							
				Berberine								
				Erythromycin								

A279	A279: PC1_G288: PC1	A279T_G288S	MIC	Chloramphenicol	/	/	/	/			
				Oxacillin	Intermediate effect (~25% of WT)	activity of neg ctrl (ΔA)					
				Linezolid	No effect	/	/	/	/		
				Minocycline							
				Novobiocin	Intermediate effect (~6% of WT)	activity of neg ctrl (ΔA)					
				Clarithromycin	Intermediate effect (~25% of WT)						
	MIC + inhibitor (NMP)	Rifampicin	No effect	/							
		Levofloxacin									
		Chloramphenicol									
		Oxacillin									
		Linezolid	No effect	/	/	/	/				
		Minocycline									
PC1	A279T	Plate dilution assay	1-Hexene			Higher growth comp to WT (10^7 cells)	/	Decreased expression comp to WT			
			1-Hexene			Higher growth comp to WT (10^7 cells)	/				
A279: PC1_L822: PC2	A279T_L822P	Growth assay	Bile Salt			No effect	/				
			1-Hexene (60 mg/L)	/	/	Higher growth comp to WT	/				
A279: PC1_Q584: PC1_L822: PC1	A279T_Q584R_L822P	Growth assay	1-Hexene (70 mg/L)								
			Styrene (100 mg/mL)								
N282	PC1	N282Y	MIC	Levofloxacin		/	/	/	/		
				Chloramphenicol							
				Oxacillin							
				Linezolid	No effect	/	/	/	/		
				Minocycline							
				Novobiocin							
	MIC + inhibitor (NMP)	Clarithromycin									
		Rifampicin									
		Levofloxacin	No effect	/							
		Chloramphenicol									
		Oxacillin	Intermediate effect (~25% of WT)	activity of neg ctrl (ΔA)							
		Linezolid			/	/	/				
G288	PN2; distal binding pocket	G288C	MIC	Novobiocin	/	/	No effect	/	/		
				Minocycline							
				Nalidixic acid							
				Erythromycin							
				Novobiocin	/	/	No effect	/	/		
				Minocycline							
	G288D	MIC	G288D	MIC	Nalidixic acid	/	/	No effect	/	/	
					Ciprofloxacin						
					SDS						
					Ciprofloxacin			Increased MIC (400% activity)	/	Comparable to WT	
					Nalidixic acid	/	/	No effect	/		
					Chloramphenicol			100% inhibition	/		
G288D	Accumulation assay	G288D	Accumulation assay	Ciprofloxacin	/	/	Decreased accumulation comp to WT	Decreased accumulation comp to neg ctrl ($\Delta AcrB$)			
				Doxorubicin			Increased accumulation comp to WT				
				Minocycline							
				Levofloxacin							
				Chloramphenicol	No effect	/			/	/	
				Oxacillin							
G288S	MIC	G288S	MIC	Linezolid							
				Minocycline							
				Novobiocin	100% inhibition	/					
				Clarithromycin	No effect	/					
				Rifampicin							
				Levofloxacin	Intermediate effect (~25% of WT)	activity of neg ctrl (ΔA)	/	/			
G288S	MIC + inhibitor (NMP)	G288S	MIC + inhibitor (NMP)	Chloramphenicol							
				Oxacillin							
				Linezolid							
				Minocycline	No effect	/			/	/	
				Novobiocin							
				Clarithromycin							
G288S	MIC	G288S	MIC	Rifampicin							
				Levofloxacin							
				Chloramphenicol							
				Oxacillin							
				Linezolid	No effect	/			/	/	
				Minocycline							

		G288M	MIC	Minocycline Novobiocin Clarithromycin Rifampicin Levofloxacin Chloramphenicol Oxacillin Linezolid Minocycline Novobiocin Clarithromycin Rifampicin	/	/	/	/	/	/	/	/	
			MIC + inhibitor (NMP)										
A294	PN2	CysLess: A294C Cys was replaced by Ala	MIC	Tetraphenylphosphonium Rhodamine 6G Berberine Erythromycin Oxacillin	/	/	No effect	/	Comparable to other mutants and ctrl	/		18223659	
		CysLess: N298C Cys was replaced by Ala	MIC	Tetraphenylphosphonium Rhodamine 6G Berberine Erythromycin Oxacillin	/	/	Intermediate effect (~12% of WT activity) Intermediate effect (~25% of WT activity)	16x activity of neg ctrl (empty vector) 8x activity of neg ctrl (empty vector) 4x activity of neg ctrl (empty vector)	Comparable to other mutants and ctrl	/		18223659	
N298	PN2	N298W	MIC on agar plates	Benzalkonium chloride Rhodamine 6G Ethidium bromide Acridiflavine Crystal violet Minocycline Cloxacillin Dequalinium Enoxacin Norfloxacin Novobiocin Erythromycin Rifampicin	/	/	Intermediate effect (~12% of WT activity) Intermediate effect (~25% of WT activity) Intermediate effect (~6% of WT activity) Intermediate effect (~25% of WT activity) Intermediate effect (~3% of WT activity) No effect Intermediate effect (~25% of WT activity) No effect Intermediate effect (~25% of WT activity) No effect	16x activity of neg ctrl (ΔacrB) 16x activity of neg ctrl (ΔacrB) 32x activity of neg ctrl (ΔacrB) 8x activity of neg ctrl (empty vector) 64x activity of neg ctrl (ΔacrB) /	Comparable to WT	Residue was identified using PDB 3AOD		29317622	
A299	PN2	CysLess: A299C Cys was replaced by Ala	MIC	Tetraphenylphosphonium Rhodamine 6G Berberine Erythromycin Oxacillin	/	/	No effect	/	Comparable to other mutants and ctrl	/		18223659	
F316	PN2	CysLess: F316C Cys was replaced by Ser CysLess: F316S Cys was replaced by Ser	Efflux assay	Cholate	/	/	No effect	/	Comparable to CysLess WT	/		17905989	
F316: PN2_Q687: between PC1 and PC2 (Interface subunit)	CysLess: F316C_Q687C Cys was replaced by Ser CysLess: F316S_Q687C Cys was replaced by Ser CysLess: F316C_A688C Cys was replaced by Ser CysLess: F316S_A688C Cys was replaced by Ser CysLess: F316C_G854C Cys was replaced by Ser CysLess: F316S_Q687C_G854C Cys was replaced by Ser	/					/	Intermediate effect (rel activity ~30% of WT activity)					Relative activity was calculated
								No effect					/
								Intermediate effect (rel activity ~10% of WT activity)					Relative activity was calculated
								No effect					/
								Intermediate effect (rel activity below 10% of WT activity)					Relative activity was calculated
								No effect					/
S319	PN2	CysLess: S319C Cys was replaced by Ala	MIC	Novobiocin	/	/	Intermediate effect	2x activity of neg ctrl	/	/		11754746	
between PN2 and TM1_ "vestibule pathway a"	Y327A	pAcBH/W3104daCrAB_neg ctrl: V		Chloramphenicol Novobiocin Erythromycin Tetracycline Minocycline Norfloxacin Tetraphenylphosphonium Acridiflavine Crystal violet Rhodamine 6G Ethidium bromide Benzalkonium Chloramphenicol	/	/	No effect Intermediate effect (~12% of WT activity) No effect 100% inhibition Intermediate effect (~25% of WT activity) No effect 100% inhibition Increased activity 200% Intermediate effect (~1.5% of WT activity) No effect 100% inhibition 100% inhibition	/ 8x activity of neg ctrl (ΔacrB) /	Comparable to WT				
Y327: between PN2 and TM1_ T329: TM2_ "vestibule pathway b"	Y327A_T329A		MIC	Novobiocin Erythromycin Tetracycline Minocycline Norfloxacin Tetraphenylphosphonium Acridiflavine Crystal violet	/	/	Intermediate effect (~12% of WT activity) Intermediate effect (~12% of WT activity) No effect 100% inhibition	2x activity of neg ctrl (ΔacrB) 4x activity of neg ctrl (ΔacrB) /	Comparable to WT				

Y327	Y327: between PN2 and TM1_T329: TM2_L137: PN2_ "vestibule pathway b"	Y327A_T329A_L137A	MIC	Rhodamine 6G	/	/	No effect	/	Comparable to WT	/	23627437									
				Ethidium bromide			100% inhibition	/												
Y327: between PN2 and TM1_T329: TM2_L137: PN2_Q569: PC1_ "vestibule pathway b"	Y327A_T329A_L137A_Q569A	Y327A_T329A_L137A_Q569A	MIC	Benzalkonium	/	/	No effect	/	Expression a bit lower cop to WT	/										
				Chloramphenicol			100% inhibition	/												
				Novobiocin			Intermediate effect (~12% of WT activity)	8x activity of neg ctrl (ΔAcrB)												
				Erythromycin			100% inhibition	/												
				Tetracycline			Intermediate effect (~25% of WT activity)	4x activity of neg ctrl (ΔAcrB)												
				Minocycline			100% inhibition	/												
				Norfloxacin			100% inhibition	/												
				Tetraphenylphosphonium			100% inhibition	/												
				Acriflavine			Intermediate effect (~6% of WT activity)	2x activity of neg ctrl (ΔAcrB)												
				Crystal violet			100% inhibition	/												
				Rhodamine 6G			Intermediate effect (~3% of WT activity)	8x activity of neg ctrl (ΔAcrB)												
				Ethidium bromide			No effect	/												
				Benzalkonium			No effect	/												
				Chloramphenicol			No effect	/												
				Novobiocin			No effect	/												
				Erythromycin			No effect	/												
Tetracycline	No effect	/																		
Minocycline	No effect	/																		
Norfloxacin	No effect	/																		
Tetraphenylphosphonium	No effect	/																		
Acriflavine	Intermediate effect (~3% of WT activity)	8x activity of neg ctrl (ΔAcrB)																		
Crystal violet	No effect	/																		
Rhodamine 6G	No effect	/																		
Ethidium bromide	No effect	/																		
Benzalkonium	No effect	/																		
T329	TM2_ "vestibule pathway b"	T329A	MIC	Chloramphenicol	/	/	No effect	/	Comparable to WT	/	23627437									
				Novobiocin			100% inhibition	/												
				Erythromycin			No effect	/												
				Tetracycline			No effect	/												
				Minocycline			No effect	/												
				Norfloxacin			100% inhibition	/												
				Tetraphenylphosphonium			100% inhibition	/												
				Acriflavine			Intermediate effect (~3% of WT activity)	4x activity of neg ctrl (ΔAcrB)												
				Crystal violet			No effect	/												
				Rhodamine 6G			No effect	/												
				Ethidium bromide			No effect	/												
				Benzalkonium			100% inhibition	/												
				I335			I335: TM2_A995: between TM11 and TM12	CysLess: I335C_A995C Cys was replaced by Ala				MIC	Erythromycin	/	/	No effect	/	Comparable to WT	/	21529338
													Novobiocin			No effect	/			
				S336			TM2	CysLess: S336C Cys was replaced by Ala				MIC	Novobiocin	/	/	No effect	/	/	/	11754746
				I337			TM2	I337A				LB Agar plate dilution assay	Fusidic acid	/	/	Intermediate effect	Higher growth comp to neg ctrl (dead mutant)	Comparable to WT	/	27982032
TPP	No effect	/																		
Erythromycin	No effect	/																		
Linezolid	No effect	/																		
Oxacillin	Intermediate effect	Higher growth comp to neg ctrl (dead mutant)																		
Dicloxacillin	No effect	/																		
Piperacillin	No effect	/																		
Cloxacillin	No effect	/																		
Fusidic acid	No effect	/																		
TPP	No effect	/																		
H338	TM1/TM2 groove	H338A	LB Agar plate dilution assay	Erythromycin	/	/	No effect	/	Comparable to WT	/	27982032									
				Linezolid			No effect	/												
				Oxacillin			No effect	/												
				Dicloxacillin			No effect	/												
				Piperacillin			No effect	/												
				Cloxacillin			No effect	/												
				Fusidic acid			No effect	/												
				TPP			No effect	/												
	H338R	LB Agar plate dilution assay	H338R	LB Agar plate dilution assay	Erythromycin	/	/	No effect	/	Comparable to WT	/	27982032								
					Linezolid			No effect	/											
					Oxacillin			No effect	/											
					Dicloxacillin			No effect	/											
					Piperacillin			No effect	/											
					Cloxacillin			No effect	/											
					Fusidic acid			No effect	/											
					TPP			No effect	/											
E339	TM2	E339A	Efflux assay	Cholate	/	/	No effect	/	Comparable to WT	/	17015667									
V340	TM2	CysLess: V340C Cys was substituted by Ala	MIC	Novobiocin	/	/	No effect	/	/	/	11754746									
V341	TM2	V341A	LB Agar plate dilution assay	Fusidic acid	/	/	No effect	/	Comparable to WT	/	27982032									
				TPP			No effect	/												
				Erythromycin			No effect	/												
				Linezolid			No effect	/												
				Oxacillin			No effect	/												
				Dicloxacillin			No effect	/												
				Piperacillin			No effect	/												
				Cloxacillin			No effect	/												
				Fusidic acid			No effect	/												
				TPP			No effect	/												
T343	TM2	T343A	Efflux assay	Cholate	/	/	No effect	/	Comparable to WT	/	17015667									

M355	TM2; cytoplasmic site	M355L	Growth assay	<i>n</i> -Butanol	/	/	Enhanced growth	/	Comparable to WT	/	23991711
L357	TM2	L357Q	Efflux rate: Hydrocarbon release rate constant/ min ⁻¹	<i>n</i> -Octane	/	/	Decreased efflux comp to WT	Higher than neg control	Comparable to WT	/	23693002
	L357; TM2_Q737; DC_M844; PC2_M987; TM11_V1028; TM12	L357Q_Q737L_M844L_M987T_V1028M		α -Pinene			No effect	/			
				<i>n</i> -Octane			Increased efflux comp to WT	/			
				α -Pinene							
L359	TM2	CysLess: L359C Cys was replaced by Ala	MIC	Novobiocin	/	/	100% inhibition	Equal activity activity	/	/	11754746
N361	between TM2 and TM3	N361A	Efflux assay	Cholate	/	/	No effect	/	Comparable to WT	/	17015667
A364	TM3	CysLess: A364C Cys was replaced by Ala	MIC	Novobiocin	/	/	Intermediate effect (~25% of WT activity)	8x activity of neg ctrl	/	/	11754746
T365	TM3	CysLess: T365C Cys was replaced by Ala	MIC	Novobiocin	/	/	Intermediate effect (~12% of WT activity)	4x activity of neg ctrl	/	/	11754746
I367	TM3	CysLess: I367C Cys was replaced by Ala	MIC	Novobiocin	/	/	100% inhibition	Equal activity activity	/	/	11754746
T369	TM3	CysLess: T369C Cys was replaced by Ala	MIC	Novobiocin	/	/	No effect	/	/	/	11754746
I370T	I370: TM3_I466; TM6_G689; between PC1 and PC2_A942; TM10	I370T_I466F_G689D_A942T	Growth assay	Isobutanol	/	/	Enhanced growth	No neg ctrl; only comp to WT-AcrB	Comparable to WT	/	23991711
				<i>n</i> -Butanol							
				<i>n</i> -Penatrol							
				<i>n</i> -Hexanol							
				<i>n</i> -Heptanol							
				<i>n</i> -Octanol							
				Ampicillin			No effect	/			
				Chloramphenicol			No effect	/			
	I370: TM3_I466; TM6_G689; between PC1 and PC2_A942; TM10_S880; TM8	I370T_I466F_G689D_A942T_S880	Growth assay	<i>n</i> -Butanol	/	/	Enhanced growth	Enhanced growth	Comparable to WT	/	
P373	TM3	P373A	Efflux assay	Cholate	/	/	Intermediate effect (60% of WT activity)	Only rel. activity was shown in the paper	Comparable to WT	/	17015667
F386	TM3; initial binding of substrate in the central cavity	F386A	MIC	Novobiocin	/	/	No effect	/	comparable to WT	/	16166543
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							
				SDS							
				Novobiocin							
				Tetracycline							
				Norflaxacin							
				Erythromycin							
				Taurocholic acid							
				Ethidium							
				Dequalinium							
				Rhodamin-6-G							
				Acriflavine							

	D408: TM4_K940: TM10	D408E_K940R	MIC	Linezolid	/	/	100% inhibition	Equal activity	Comparable to WT	/	19425588
				Tetraphenylphosphonium			Intermediate effect (~12% of WT activity)	8x activity of neg ctrl (empty vector)			
				Oxacillin			No effect	/			
				Rhodamine 6G			Intermediate effect (~25% of WT activity)	8x activity of neg ctrl (empty vector)			
				Berberine			Intermediate effect (~12% of WT activity)	2x activity of neg ctrl (empty vector)			
				Erythromycin			Intermediate effect (~25% of WT activity)	4x activity of neg ctrl (empty vector)			
				Linezolid							
		D408E_K940H		Tetraphenylphosphonium			100% inhibition	Equal activity			
				Oxacillin							
				Rhodamine 6G							
				Berberine							
				Erythromycin							
				Linezolid							
	TM4	D408A	Complementation assay	Ciprofloxacin	/	/	100% inhibition	Equal activity	Comparable to WT	/	28497315
		D408N									
I410	TM4	I410A	Efflux assay	Cholate	/	/	Intermediate effect (~60% of WT activity)	Only rel. activity was shown in the paper	Comparable to WT	/	17015667
V411	TM4	V411A	Complementation assay	Ciprofloxacin	/	/	No effect	Higher growth	Comparable to WT	/	28497315
		V411T					100% inhibition	Equal activity			
E414	Between TM4 and TM5 (from D407/D408/K922 triad to cytoplasm)	E414A	Efflux assay	Cholate	/	/	Intermediate effect (~50% of WT activity)	Only rel. activity was shown in the paper	Comparable to WT	/	17015667
		E414A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	/	19425588
				Oxacillin							
				Rhodamine 6G							
				Berberine							
				Erythromycin							
				Linezolid			Intermediate effect (~12% of WT activity)	4x activity of neg ctrl (empty vector)			
E417	Between TM4 and TM5 (from D407/D408/K922 triad to cytoplasm)	E417A	Efflux assay	Cholate	/	/	No effect	/	Comparable to WT	/	17015667
		E414Q	MIC	Tetraphenylphosphonium	/	/	No effect	/	Comparable to WT	/	19425588
				Oxacillin							
				Rhodamine 6G							
				Berberine							
				Erythromycin							
				Linezolid							
R418	Between TM4 and TM5 (from D407/D408/K922 triad to cytoplasm)	R418A	Efflux assay	Cholate	/	/	No effect	/	Comparable to WT	/	17015667
		R418A	MIC	Tetraphenylphosphonium	/	/	No effect	/	Lower than other mutants	/	19425588
				Oxacillin							
				Rhodamine 6G							
				Berberine							
				Erythromycin							
				Linezolid							
A421	TM4	CysLess: A421C Cys was replaced by Ala	MIC	Novobiocin	/	/	No effect	/	/	/	11754746
F453	TM5; close to proton relay network	F453C	MIC	Erythromycin	/	/	No effect	/	/	/	26240069
				Novobiocin	/	/					
				Minocycline	/	/					
				Nalidixic acid	/	/	100% inhibition	Equal activity			
F458	TM5	F458A	MIC (pos. Ctrl. AcrAB wt; neg. Ctrl. Empty vector)	Novobiocin	/	/	no effect	-	like wt (pAcrAB)	no	16166543
				Tetracycline							
				Norfloxacin							
				Erythromycin							
				Taurochloric acid							
				Ethidium							
				Dequalinium							
				Rhodamine 6G							
				Acriflavine							
				SDS							
	TM5 and loop TM5/6 (periplasm)	F458A_F459A	MIC (pos. Ctrl. AcrAB wt; neg. Ctrl. Empty vector)	Novobiocin	/	/	no effect	-	not determined	no	16166543
				Tetracycline							
				Norfloxacin							
				Erythromycin							
				Taurochloric acid							
				Ethidium							
				Dequalinium							
				Rhodamine 6G							
				Acriflavine							
				SDS							
F459	TM6	F459A	MIC (pos. Ctrl. AcrAB wt; neg. Ctrl. Empty vector)	Novobiocin	/	/	no effect	-	like wt (pAcrAB)	no	16166543
				Tetracycline							
				Norfloxacin							
				Erythromycin							
				Taurochloric acid							
				Ethidium							
				Dequalinium							
				Rhodamine 6G							
				Acriflavine							
				SDS							

S462	Loop TM5-6	S462C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
		S462K	Growth curves	Erythromycin	/	/	100% inhibition (behavior more like empty vector)	–	not determined	no	22121023
Efflux transport	Doxorubicin		/	/	100% inhibition (no efflux of doxo, quenching of fluorescence (like neg. Ctrl = vector))	–	not determined				
A465	TM6	A465C AcrB CL (=Cys-less, replaced by A)	MIC(pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
Q469	TM6	Q469C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
	TM6	Q469A_T473S	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrB _{His})	no	17015667
T473	TM6	T473A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrB _{His})	no	17015667
S481	TM6	S481A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	intermed. effect (40% activity)		like wt (pSAcrB _{His})	no	17015667
				TPP	/	/	no effect	–	not determined	no	19425588
C493	TM6	C493A_C887A = CL Mutant	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Oxacillin	/	/	no effect	–			
				Rhodamine 6G	/	/	intermed. effect (~25% activity)	16x			
L486	TM6	L486W	MIC (pos. Ctrl AcrB wt; no neg. Ctrl.)	Berberine	/	/	intermed. effect (~25% activity)	4x	not determined	no	26240069
				Erythromycin	/	/	no effect	–			
L486	TM6	L486W_F610A	MIC (pos. Ctrl AcrB wt; neg. Ctrl. ΔAcrAB)	Linezolid	/	/	no effect	–	not determined	no	26240069
				Erythromycin	/	/	no effect	–			
L486	TM6	L486W_F615A	MIC (pos. Ctrl AcrB wt; neg. Ctrl. ΔAcrAB)	Novobiocin	/	/	no effect	–	not determined	no	26240069
				Minocycline	/	/	intermed. Effect (25% activity)	16x			
T489	TM6	T489A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrB _{His})	no	17015667
T495	Loop TM6-7	T495A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrB _{His})	no	17015668
V501	Loop TM6-7	V501C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
Y527	Loop Tm6-7	Y527A	Plate Dilution Assay (pos. Ctrl AcrB wt; neg. Ctrl. D407N mutant)	Ciprofloxacin (5 ng/mL)	/	/	no effect (behavior like wt)		like wt (pET21a_acrB)	no	28497315
					/	/	100% inhibition		like wt (pET21a_acrB)	no	
					/	/	intermed. Effekt (less growth)		like wt (pET21a_acrB)	no	
					/	/	no effect (behavior like wt)		like wt (pET21a_acrB)	no	
					/	/	100% inhibition		like wt (pET21a_acrB)	no	
					/	/	no effect (behavior like wt)		like wt (pET21a_acrB)	no	
					/	/	no effect (behavior like wt)		like wt (pET21a_acrB)	no	
F556	TM7	F556A	MIC (pos. Ctrl. AcrAB wt; neg. Ctrl. Empty vector)	Novobiocin	/	/	no effect	–	not determined	no	16166543
				Tetracycline	/	/	no effect	–			
				Norfloxacin	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
				Tetracycline	/	/	no effect	–			
				Ethidium	/	/	no effect	–			
				Dequalinium	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				Acridiflavine	/	/	no effect	–			

R558	TM7	R558C AcrB CL (=Cyc-less, replaced by A)	MIC(pos. Ctrl AcrB CL; neg. Ctrl. Empty vector)	SDS	/	/	no effect	–	not determined	no	18223659
				TPP	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				Berberine	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
		Oxacillin	/	/	no effect	–					
		R558C_E839C AcrB CL (=Cyc-less, replaced by A)	MIC (pos. Ctrl AcrB CL; neg. Ctrl. Empty vector)	TPP	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				Berberine	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
Oxacillin	/			/	no effect	–					
P560	Loop TM7-PC1	P560A	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N mutant)	Ciprofloxacin (5 ng/ml)	/	/	intermed. Effekt (less growth)	–	like wt (pET21a_acrB)	no	28497315
		P560A_P565A		/	/	intermed. Effekt (less growth)	–	like wt (pET21a_acrB)	no		
S562	Loop TM7-PC1	S562C AcrB CL (=Cyc-less, replaced by A)	MIC (pos. Ctrl AcrB CL; neg. Ctrl. Empty vector)	TPP	/	/	no effect	–	not determined	no	18223659
				Rhodamine 6G	/	/	no effect	–			
				Berberine	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
				Oxacillin	/	/	no effect	–			
		S562C_T837C AcrB CL (=Cyc-less, replaced by A)	Efflux transport	TPP	/	/	100% Inhibition	0.016x			
				Rhodamine 6G	/	/	intermed. Effekt (25% activity)	16x			
				Berberine	/	/	intermed. Effekt (25% activity)	4x			
				Erythromycin	/	/	intermed. Effekt (25% activity)	5x			
				Oxacillin	/	/	intermed. Effekt (25% activity)	16x			
S562P	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N mutant)	Ciprofloxacin (5ng/ml)	NPN (10 µM)	/	/	no effect (quenching of fluorescence, like pos. Ctrl.)	–	higher than pos. Ctrl. (pET24a_AcrB_cl)	no	28497315	
			NPN (10 µM) + DL-DTT (10 mM)	/	/	intermed. Effekt (less quenching of fluorescence compared to pos Ctrl. and crosslinked AcrB (no negt. Ctrl))	–	higher than pos. Ctrl. (pET24a_AcrB_cl)	no		
			/	/	no effect (behavior like positive control)	–	like wt (pET21a_acrB)	no			
P565	Loop TM7-PC1	P565A	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N mutant)	Ciprofloxacin (5ng/ml)	/	/	intermed. Effekt (less growth)	–	like wt (pET21a_acrB)	no	28497315
					/	/	intermed. Effekt (less growth)	–	like wt (pET21a_acrB)	no	
					/	/	intermed. Effekt (less growth)	–	like wt (pET21a_acrB)	no	
D566	Loop TM7-PC1	D566A	MIC (pos. Ctrl. AcrAB wt; neg. Ctrl. Empty vector)	Novobiocin	/	/	no effect	–	not determined	no	16166543
				Tetracycline	/	/	no effect	–			
				Norfloxacin	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
				Tetracycline	/	/	no effect	–			
				Ethidium	/	/	no effect	–			
				Dequalinium	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				Acriflavine	/	/	no effect	–			
				SDS	/	/	no effect	–			
D566C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	D566C AcrB CL (=Cyc-less, replaced by S)	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Erythromycin	/	/	100% Inhibition	2x	not determined	no	24551234
				Novobiocin	/	/	100% Inhibition	2x	not determined	no	
				Cholate	/	/	no effect (90% activity)	–	not determined	no	
				Cholate	/	/	intermed. Effekt (10%activity)	–	not determined	no	
				Cholate	/	/	no effect (95% activity)	–	not determined	no	
D566S	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	D566S AcrB CL (=Cyc-less, replaced by S)	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholate	/	/	no effect (80% activity)	–	not determined	no	17905989
				Cholate	/	/	no effect (80% activity)	–	not determined	no	
				Cholate	/	/	no effect (80% activity)	–	not determined	no	
				Cholate	/	/	no effect (80% activity)	–	not determined	no	
				Cholate	/	/	no effect (80% activity)	–	not determined	no	
Q569R	Loop TM7-PC1	Q569R	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Azteonam	/	/	no effect	–	like wt (pTHacrAB-His)	no	17905989
				Cabencillin	/	/	no effect	–			
				Sulbencillin	/	/	no effect	–			
				Oxacillin	/	/	no effect	–			
				Cloxacillin	/	/	no effect	–			
				Nafcillin	/	/	no effect	–			
				Cefamandole	/	/	100% Inhibition	2x			
				Novobiocin	/	/	no effect	–			
				Erythromycin	/	/	no effect	–			
				Tetracycline	/	/	no effect	–			
				Minocycline	/	/	no effect	–			
				Nalidixic acid	/	/	no effect	–			
				Norfloxacin	/	/	no effect	–			
				Enoxacin	/	/	no effect	–			
				Trimethoprim	/	/	no effect	–			
				Triclosan	/	/	no effect	–			
				Acriflavine	/	/	intermed. Effekt (25% activity)	32x			

Q569

PC1

Q569R_E673G
Q569R_I626R
Q569R_I626R_E673G

MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. native AcrAB, neg. Ctrl. Strain ΔacrD and strain has no acrB)

Crystal violet	/	/	no effect	–
Ethidium	/	/	no effect	–
Rhodamine 6G	/	/	no effect	–
TPP	/	/	no effect	–
Benzalkonium	/	/	no effect	–
SDS	/	/	no effect	–
Deoxycholate	/	/	no effect	–
Azteonam	/	/	no effect	–
Cabencillin	/	/	400% higher activity	–
Subencillin	/	/	400% higher activity	–
Oxacillin	/	/	no effect	–
Cloxacillin	/	/	no effect	–
Naficillin	/	/	no effect	–
Cefamandole	/	/	no effect	–
Novobiocin	/	/	no effect	–
Erythromycin	/	/	no effect	–
Tetracycline	/	/	no effect	–
Minocycline	/	/	no effect	–
Nalidixic acid	/	/	no effect	–
Norfloxacin	/	/	no effect	–
Enoxacin	/	/	no effect	–
Trimethoprim	/	/	no effect	–
Triclosan	/	/	no effect	–
Acriflavine	/	/	no effect	–
Crystal violet	/	/	no effect	–
Ethidium	/	/	no effect	–
Rhodamine 6G	/	/	no effect	–
TPP	/	/	no effect	–
Benzalkonium	/	/	no effect	–
SDS	/	/	no effect	–
Deoxycholate	/	/	no effect	–
Azteonam	/	/	200% higher activity	–
Carbencillin	/	/	400% higher activity	–
Subencillin	/	/	800% higher activity	–
Oxacillin	/	/	no effect	–
Cloxacillin	/	/	no effect	–
Naficillin	/	/	no effect	–
Cefamandole	/	/	no effect	–
Novobiocin	/	/	no effect	–
Erythromycin	/	/	100% inhibition	2x
Tetracycline	/	/	no effect	–
Minocycline	/	/	intermed. Effect (25% activity)	4x
Nalidixic acid	/	/	no effect	–
Norfloxacin	/	/	100% inhibition	2x
Enoxacin	/	/	100% inhibition	2x
Trimethoprim	/	/	no effect	–
Triclosan	/	/	no effect	–
Acriflavine	/	/	intermed. Effect (12.5% activity)	16x
Crystal violet	/	/	no effect	–
Ethidium	/	/	intermed. Effect (6% activity)	8x
Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	128x
TPP	/	/	intermed. Effect (12.5% activity)	64x
Benzalkonium	/	/	no effect	–
SDS	/	/	no effect	–
Deoxycholate	/	/	no effect	–
Azteonam	/	/	3200% higher activity	–
Carbencillin	/	/	3200% higher activity	–
Subencillin	/	/	12800% higher activity	–
Oxacillin	/	/	no effect	–
Cloxacillin	/	/	no effect	–
Naficillin	/	/	no effect	–
Cefamandole	/	/	400% higher activity	–
Novobiocin	/	/	no effect	–
Erythromycin	/	/	100% inhibition	2x
Tetracycline	/	/	no effect	–
Minocycline	/	/	no effect	–
Nalidixic acid	/	/	no effect	–
Norfloxacin	/	/	100% inhibition	2x
Enoxacin	/	/	100% inhibition	2x
Trimethoprim	/	/	no effect	–
Triclosan	/	/	no effect	–
Acriflavine	/	/	intermed. Effect (6% activity)	8x
Crystal violet	/	/	intermed. Effect (25% activity)	16x
Ethidium	/	/	intermed. Effect (6% activity)	8x
Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	128x
TPP	/	/	intermed. Effect (6% activity)	32x
Benzalkonium	/	/	no effect	–
SDS	/	/	no effect	–
Deoxycholate	/	/	no effect	–
Azteonam	/	/	3200% higher activity	–

like wt (pTHacrAB-His)

no

like wt (pTHacrAB-His)

no

like wt (pTHacrAB-His)

no

24558035

		Q569R_I626R_M662R_E673G		Carbenicillin / / 3200% higher activity -				
				Subbenicillin / / 12800% higher activity -				
				Oxacillin / / no effect -				
				Cloxacillin / / no effect -				
				Nafcillin / / no effect -				
				Cefamandole / / 400% higher activity -				
				Novobiocin / / no effect -				
				Erythromycin / / 100% inhibition 2x				
				Tetracycline / / no effect -				
				Minocycline / / intermed. Effect (25% activity) 4x				
				Nalidixic acid / / no effect -				
				Norfloxacin / / 100% inhibition 2x				
				Enoxacin / / 100% inhibition 2x				
				Trimethoprim / / no effect -				
				Triclosan / / no effect -				
				Acriflavine / / intermed. Effect (6% activity) 8x				
				Crystal violet / / intermed. Effect (25% activity) 16x				
				Ethidium / / intermed. Effect (3% activity) 4x				
				Rhodamine 6G / / intermed. effect (6% activity) 64x				
				TPP / / intermed. effect (6% activity) 32x				
				Benzalkonium / / no effect -				
				SDS / / no effect -				
				Deoxycholate / / no effect -				
				Azteonam / / 1600% higher activity -				
				Carbenicillin / / 3200% higher activity -				
				Subbenicillin / / 6400% higher activity -				
				Oxacillin / / no effect -				
				Cloxacillin / / no effect -				
				Nafcillin / / no effect -				
				Cefamandole / / no effect -				
				Novobiocin / / no effect -				
				Erythromycin / / 100% inhibition 2x				
				Tetracycline / / no effect -				
				Minocycline / / no effect -				
				Nalidixic acid / / no effect -				
				Norfloxacin / / 100% inhibition 2x				
				Enoxacin / / 100% inhibition 2x				
				Trimethoprim / / no effect -				
				Triclosan / / no effect -				
				Acriflavine / / intermed. Effect (6% activity) 8x				
				Crystal violet / / intermed. Effect (25% activity) 16x				
				Ethidium / / intermed. Effect (6% activity) 8x				
				Rhodamine 6G / / intermed. Effect (6% activity) 64x				
				TPP / / intermed. Effect (6% activity) 32x				
				Benzalkonium / / no effect -				
				SDS / / no effect -				
				Deoxycholate / / no effect -				
				Azteonam / / 1600% higher activity -				
				Carbenicillin / / 3200% higher activity -				
				Subbenicillin / / 6400% higher activity -				
				Oxacillin / / no effect -				
				Cloxacillin / / no effect -				
				Nafcillin / / no effect -				
				Cefamandole / / 100% higher activity -				
				Novobiocin / / no effect -				
				Erythromycin / / 100% inhibition 2x				
				Tetracycline / / no effect -				
				Minocycline / / intermed. Effect (25% activity) 4x				
				Nalidixic acid / / no effect -				
				Norfloxacin / / 100% inhibition 2x				
				Enoxacin / / 100% inhibition 2x				
				Trimethoprim / / no effect -				
				Triclosan / / no effect -				
				Acriflavine / / intermed. Effect (6% activity) 8x				
				Crystal violet / / intermed. Effect (25% activity) 16x				
				Ethidium / / intermed. Effect (6% activity) 8x				
				Rhodamine 6G / / intermed. Effect (6% activity) 64x				
				TPP / / intermed. Effect (3% activity) 16x				
				Benzalkonium / / no effect -				
				SDS / / no effect -				
				Deoxycholate / / no effect -				
G570	PC1	G570C_A627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Erythromycin / / no effect -		not determined	no	21529338
F572	PC1	F572C_A627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Erythromycin / / no effect -		not determined	no	21529338
T574	PC1	T574C_A627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Erythromycin / / no effect -		not determined	no	21529338
				Novobiocin / / no effect -				
V576	PC1	V576C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin / / 100% inhibition -		was expressed (no pos. Ctrl on gel)	no	11754746

		L578C_A627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Erythromycin	/	/	no effect	—	not determined	no	21529338
L578	PC1	L578C_A627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Novobiocin	/	/	no effect	—	not determined	no	21529338
				Erythromycin	/	/	no effect	—			
				Novobiocin	/	/	no effect	—			
				TPP	/	/	no effect	—			
				Rhodamine 6G	/	/	no effect	—			
				Berberine	/	/	no effect	—			
				Erythromycin	/	/	no effect	—			
				Oxacillin	/	/	no effect	—			
				Acriflavine	/	/	no effect	—			
				Erythromycin	/	/	no effect	—			
				Novobiocin	/	/	no effect	—			
				Rhodamine 6G	/	/	no effect	—			
				TPP	/	/	no effect	—			
				1-hexene saturated atmosphere	/	/	10 ⁷ cells: better than wt	—			
				10 ⁶ cells: better than wt	/	/	—	—			
				Growth with different conc. of substrate	/	/	no effect (behavior like wt (decreased OD with increased concentration))	—			
				Styrene (0-140 mg/L)	/	/	—	—			
				Bile Salt (0 and 5 g/L)	/	/	no effect (behavior like wt (same OD))	—			
				TPP	/	/	no effect	—			
				Rhodamine 6G	/	/	no effect	—			
				Berberine	/	/	no effect	—			
				Erythromycin	/	/	no effect	—			
				Oxacillin	/	/	no effect	—			
				Linezolid	no effect	—	/	/			
				Chloramphenicol	intermed. Effect (25% activity)	4x	/	/			
				Cefuroxime	no effect	—	/	/			
				Oxacillin	no effect	—	/	/			
				Clindamycin	no effect	—	/	/			
				Tetracycline	no effect	—	/	/			
				Tigecycline	no effect	—	/	/			
				Levofloxacin	no effect	—	/	/			
				Novobiocin	no effect	—	/	/			
				Rifampicin	no effect	—	/	/			
				Clarithromycin	400% higher activity	—	/	/			
				Erythromycin	no effect	—	/	/			
				Azithromycin	no effect	—	/	/			
				Linezolid	intermed. Effect (6% activity)	4x	/	/			
				Chloramphenicol	100% inhibition	—	/	/			
				Cefuroxime	intermed. Effect (6% activity)	4x	/	/			
				Oxacillin	intermed. Effect (25% activity)	128x	/	/			
				Clindamycin	intermed. Effect (25% activity)	32x	/	/			
				Tetracycline	100% inhibition	—	/	/			
				Tigecycline	100% inhibition	2x	/	/			
				Levofloxacin	intermed. Effect (25% activity)	4x	/	/			
				Novobiocin	intermed. Effect (12.5% activity)	256x	/	/			
				Rifampicin	no effect	—	/	/			
				Clarithromycin	400% higher activity	—	/	/			
				Erythromycin	no effect	—	/	/			
				Azithromycin	no effect	—	/	/			
				Linezolid	no effect	—	/	/			
				Chloramphenicol	no effect	—	/	/			
				Cefuroxime	no effect	—	/	/			
				Oxacillin	no effect	—	/	/			
				Clindamycin	no effect	—	/	/			
				Tetracycline	no effect	—	/	/			
				Tigecycline	no effect	—	/	/			
				Levofloxacin	no effect	—	/	/			
				Novobiocin	intermed. Effect (25% activity)	128x	/	/			
				Rifampicin	no effect	—	/	/			
				Clarithromycin	400% higher activity	—	/	/			
				Erythromycin	no effect	—	/	/			
				Azithromycin	no effect	—	/	/			
				Growth Curves	Erythromycin (32mg/ml)	/	/	100% Inhibition (growth like neg. Ctrl. (=empty vector))	—	not determined	
				Efflux transport	Doxorubicin	/	/	100% Inhibition (no efflux of doxo, quenching of fluorescence (like neg. Ctrl = vector))	—	not determined	
					Erythromycin	/	/	100% Inhibition	2x		
					Novobiocin	/	/	intermed. Effect (12.5% activity)	8x		
					Minocycline	/	/	100% Inhibition	2x		
					Nalidixic acid	/	/	100% Inhibition	2x		
					Ciprofloxacin	/	/	100% Inhibition	—		
					SDS	/	/	no effect	—		
					NPN (10 µM)	/	/	t _{efflux50%} (sec) 3x higher like wt	—		
					Nitrocefin (conc. ?)	/	/	intermed. Effekt (V _m = 3.09 ± 1.20 nmol/mg/s (wt: 0.0039 ± 0.0008))	no neg. Ctrl.		
					Oxacillin	/	/	intermed. Effect (25% activity)	32x		
					Doxorubicin	/	/	no effect	—		

F610	PC1	F610A	MIC (pos. Ctrl. AcrB wt; neg. ctrl. acrB:rpst-neo - knockout)	Novobiocin	/	/	intermed. Effect (6% activity)	8x	like wt	no	18849422
				Clarithromycin	/	/	intermed. Effect (3% activity)	4x			
				Erythromycin	/	/	intermed. Effect (12.5% activity)	16x			
				Azithromycin	/	/	intermed. Effect (3% activity)	4x			
				Clindamycin	/	/	intermed. Effect (12.5% activity)	8x			
				Pyronin Y	/	/	intermed. Effect (6% activity)	4x			
				Minocycline	/	/	100% inhibition	2x			
				Ethidium	/	/	no effect	—			
				Levofloxacin	/	/	100% inhibition	2x			
				Ciprofloxacin	/	/	100% inhibition	2x			
				Propidium iodide	/	/	no effect	—			
				PAβN	/	/	100% inhibition	2x			
		Chloramphenicol	/	/	100% inhibition	2x					
		Tetracycline	/	/	100% inhibition	—					
		Efflux transport	Ethidium (2.5 μM)	/	/	no effect (like wt)	—				
			PAβN (200 μM)	/	/	no effect (like wt)	—				
		Efflux transport	Nitrocefin (conc.?)	/	/	intermed. Effect (Cp (at 175 μM C ₅₀) 38.5 μM (wt 19.5 μM))	no neg. Ctrl.				
				/	/	intermed. Effect (K _{0.5} 1030 ± 400 μM (wt 3.8 ± 1.0 μM))	no neg. Ctrl.				
				/	/	intermed. Effect (vmax 3.1 ± 1.2 nmol/s/mg (wt 0.039 ± 0.006 nmol/s/mg))	no neg. Ctrl.				
				/	/	intermed. Effect (Cp (at 1 μM CD) 154 μM (wt 268 μM))	no neg. Ctrl.				
				/	/	intermed. Effect (K _{0.5} 92 ± 16 μM (wt 176 ± 19 μM))	no neg. Ctrl.				
				/	/	intermed. Effect (vmax 110 ± 41 nmol/s/mg (wt 82 ± 16 nmol/s/mg))	no neg. Ctrl.				
		Efflux transport (determ. of EHT=efflux half times over 400s)	1,2'-DNA: 0.25 μM	/	/	intermed. Effect (73 ± 24 s (wt: 20 ± 5 s))	no neg. Ctrl.				
			1,2'-DNA: 1 μM	/	/	intermed. Effect (67 ± 8.3 s (wt: 19 ± 1.5 s))	no neg. Ctrl.				
1,2'-DNA: 4 μM	/		/	intermed. Effect (97 ± 9.6 s (wt: 24 ± 2.5 s))	no neg. Ctrl.						
1,2'-DNA: 16 μM	/		/	intermed. Effect (118 ± 5.9 s (wt: 27 ± 2.9 s))	no neg. Ctrl.						
	1,2'-DNA: 64 μM	/	/	intermed. Effect (146 ± 16.8 s (wt: 37 ± 5.2 s))	no neg. Ctrl.						
F610A	Fold decrease in MIC (compared with AcrB in E. coli K-12 strain 3-AG100)	Oxacillin	intermed. Effect (4)	no neg. Ctrl.	/	/	not determined	no	25114133		
		Novobiocin	intermed. Effect (16)	no neg. Ctrl.	/	/					
		Clarithromycin	intermed. Effect (32)	no neg. Ctrl.	/	/					
		Erythromycin	intermed. Effect (8)	no neg. Ctrl.	/	/					
		Linezolid	intermed. Effect (4)	no neg. Ctrl.	/	/					
		Ethidium	intermed. Effect (2)	no neg. Ctrl.	/	/					
		Levofloxacin	intermed. Effect (8)	no neg. Ctrl.	/	/					
		Ciprofloxacin	intermed. Effect (8)	no neg. Ctrl.	/	/					
		Chloramphenicol	intermed. Effect (4)	no neg. Ctrl.	/	/					
		Tetracycline	intermed. Effect (4)	no neg. Ctrl.	/	/					
		Linezolid	intermed. Effect (6% activity)	4x	/	/					
		Chloramphenicol	100% inhibition	—	/	/					
Cefuroxime	intermed. Effect (6% activity)	4x	/	/							
Oxacillin	intermed. Effect (25% activity)	128x	/	/							
Clindamycin	intermed. Effect (25% activity)	32x	/	/							
Tetracycline	100% inhibition	—	/	/							
Tigecycline	100% inhibition	2x	/	/							
Levofloxacin	intermed. Effect (25% activity)	4x	/	/							
Novobiocin	intermed. Effect (12.5% activity)	128x	/	/							
Rifampicin	no effect	—	/	/							
Clarithromycin	400% higher activity	—	/	/							
Erythromycin	no effect	—	/	/							
Azithromycin	no effect	—	/	/							
G614P	G614P	Rhodamine 6G	/	/	100% inhibition	2x	like wt (pET24 _{acrB})	no			
		SDS	/	/	100% inhibition	—					
		TPP	/	/	100% inhibition	—					
		Erythromycin	/	/	100% inhibition	—					
		Oxacillin	/	/	100% inhibition	—					
		Ethidium	/	/	100% inhibition	2x					
		Linezolid	/	/	100% inhibition	—					
		Novobiocin	/	/	100% inhibition	—					
		Rhodamine 6G	/	/	100% inhibition	2x					
		SDS	/	/	intermed. Effect (1.5% activity)	8x					
		TPP	/	/	intermed. Effect (25% activity)	16x					
		Erythromycin	/	/	100% inhibition	2x					
G614P_F615A	G614P_F615A	Oxacillin	/	/	intermed. Effect (6% activity)	8x	like wt (pET24 _{acrB})	no			
		Ethidium	/	/	intermed. Effect (12.5% activity)	4x					
		Linezolid	/	/	100% inhibition	—					
		Novobiocin	/	/	100% inhibition	2x					
		Rhodamine 6G	/	/	100% inhibition	2x					
		SDS	/	/	intermed. Effect (3% activity)	16x					
G614P_F617A	G614P_F617A	TPP	/	/	no effect	—	like wt (pET24 _{acrB})	no			
		Erythromycin	/	/	no effect	—					
			/	/							

Strain	PC1 (sw-loop)	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)		MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)		WT (pET24a_acrB)	no	28987732 + supplement						
			Drug	Effect	Activity	Activity									
G614	PC1 (sw-loop)	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Oxacillin	/	/	intermed. Effect (12.5% activity)	16x	like wt (pET24a_acrB)	no	28987732 + supplement					
			Ethidium	/	/	intermed. Effect (25% activity)	8x								
			Linezolid	/	/	100% inhibition	—								
			Novobiocin	/	/	100% inhibition	—								
			Rhodamine 6G	/	/	100% inhibition	—								
			SDS	/	/	100% inhibition	—								
			TPP	/	/	100% inhibition	—								
			Erythromycin	/	/	100% inhibition	—								
			Oxacillin	/	/	100% inhibition	—								
			Ethidium	/	/	100% inhibition	—								
			Linezolid	/	/	100% inhibition	—								
			Novobiocin	/	/	100% inhibition	—								
			Rhodamine 6G	/	/	100% inhibition	—								
			SDS	/	/	100% inhibition	2x								
			TPP	/	/	100% inhibition	2x								
			Erythromycin	/	/	100% inhibition	—								
			Oxacillin	/	/	100% inhibition	—								
Ethidium	/	/	100% inhibition	—											
Linezolid	/	/	100% inhibition	—											
Novobiocin	/	/	100% inhibition	—											
Rhodamine 6G	/	/	100% inhibition	2x											
SDS	/	/	100% inhibition	—											
TPP	/	/	100% inhibition	2x											
Erythromycin	/	/	100% inhibition	—											
Oxacillin	/	/	100% inhibition	—											
Ethidium	/	/	100% inhibition	—											
Linezolid	/	/	100% inhibition	—											
Novobiocin	/	/	100% inhibition	—											
Rhodamine 6G	/	/	100% inhibition	—											
SDS	/	/	100% inhibition	—											
TPP	/	/	100% inhibition	—											
Erythromycin	/	/	100% inhibition	—											
Oxacillin	/	/	100% inhibition	—											
Ethidium	/	/	100% inhibition	—											
Linezolid	/	/	100% inhibition	2x											
Novobiocin	/	/	100% inhibition	—											
Rhodamine 6G	/	/	100% inhibition	—											
SDS	/	/	100% inhibition	—											
TPP	/	/	100% inhibition	—											
Erythromycin	/	/	100% inhibition	—											
Oxacillin	/	/	100% inhibition	—											
Ethidium	/	/	100% inhibition	—											
Linezolid	/	/	100% inhibition	—											
Novobiocin	/	/	100% inhibition	—											
Growth Curves	Erythromycin (32mg/ml)	/	/	100% Inhibition (growth like neg. Ctrl. (=empty vector))	—	not determined	no	22121023							
Efflux transport	Doxorubicin	/	/	100% Inhibition (no efflux of doxo, quenching of fluorescence (like neg. Ctrl = vector))	—	not determined									
G614	PC1 (sw-loop)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. ΔacrAB)	Erythromycin	/	/	100% inhibition	—	like wt (pACYC184 AcrB)	no	26240069					
			Novobiocin	/	/	100% inhibition	—								
			Minocycline	/	/	100% inhibition	2x								
			Nalidixic acid	/	/	100% inhibition	—								
			Rhodamine 6G	/	/	no effect	—								
			SDS	/	/	no effect	—								
			TPP	/	/	increased activity (200%)	—								
			Erythromycin	/	/	no effect	—								
			Oxacillin	/	/	intermed. Effect (25% activity)	32x								
			Ethidium	/	/	no effect	—								
			Linezolid	/	/	no effect	—								
			Novobiocin	/	/	intermed. Effect (25% activity)	4x								
			F615A	Efflux transport	Nitrocefin (conc.?)	/	/				no effect (Cp (at 175 μM C ₀) 24.1 μM (wt 19.5 μM))	—	not determined	no	27789287
						/	/				no effect (K _{0.5} 4.3 ± 1.9 μM (wt 3.8 ± 1.0 μM))	—			
						/	/				no effect (v _{max} 0.03 ± 0.002 nmol/s/mg (wt 0.039 ± 0.006 nmol/s/mg))	—			
						/	/				intermed. Effect (K _{0.5} 106 ± 50 μM (wt 176 ± 19 μM))	no negativ control			
						/	/				intermed. Effect (v _{max} 217 ± 148 nmol/s/mg (wt 82 ± 16 nmol/s/mg))	no negativ control			
/	/	intermed. Effect (hill-coeff. 1.8 ± 0.7 (wt 1.7 ± 0.1))				no negativ control									
Efflux transport (determ. of EHT=efflux half times over 400s)	1,2'-DNA: 0.25 μM	/	/	intermed. Effect (37 ± 7.1 s (wt: 20 ± 5 s))	no negativ control	not determined	no	21698261							
		/	/	intermed. Effect (40 ± 10.6 s (wt: 19 ± 1.5 s))	no negativ control										
		/	/	intermed. Effect (48 ± 6.7 s (wt: 24 ± 2.5 s))	no negativ control										
		/	/	intermed. Effect (48 ± 5.1 s (wt: 27 ± 2.9 s))	no negativ control										
		/	/	intermed. Effect (55 ± 1.2 s (wt: 37 ± 5.2 s))	no negativ control										
MIC (pos. Ctrl. AcrB wt; neg. ctrl. acrB:rpSL-neo - knockout)	Oxacillin	intermed. Effect (25% activity)	128x	/	/	like wt	no	18849422							
		Novobiocin	intermed. Effect (12.5% activity)						16x						
		Clarithromycin	intermed. Effect (6% activity)						8x						
		Erythromycin	intermed. Effect (6% activity)						16x						
		Ethidium (2.5 μM)	no effect (like wt)						—						
Efflux transport	PAβN (200 μM)	no effect (like wt)	—	/	/	like wt	no								
		Rhodamine 6G	100% inhibition						2x						
		SDS	100% inhibition						2x						
		TPP	100% inhibition						2x						
		Erythromycin	100% inhibition						—						

F615	PC1 (sw-loop)			Oxacillin	/	/	intermed. Effect (3% activity)	4x	(pET24a_acrB)	™					
				Ethidium			100% inhibition	–							
				Linezolid			100% inhibition	–							
				Novobiocin			100% inhibition	–							
				Rhodamine 6G			no effect	–							
				SDS			no effect	–							
				TPP			no effect	–							
				Erythromycin	/	/	no effect	–	like wt (pET24a_acrB)	no					
Oxacillin			no effect	–											
Ethidium			intermed. Effect (25% activity)	8x											
Linezolid			no effect	–											
Novobiocin			intermed. Effect (25% activity)	4x											
Rhodamine 6G			intermed. Effect (25% activity)	8x											
SDS			intermed. Effect (6% activity)	32x											
TPP			intermed. Effect (25% activity)	16x											
Erythromycin	/	/	100% inhibition	2x	like wt (pET24a_acrB)	no									
Oxacillin			intermed. Effect (25% activity)	32x											
Ethidium			intermed. Effect (12.5% activity)	4x											
Linezolid			100% inhibition	2x											
Novobiocin			100% inhibition	2x											
Rhodamine 6G			no effect	–											
SDS			intermed. Effect (3% activity)	16x											
TPP			increased activity (200%)	–											
Erythromycin	/	/	no effect	–	like wt (pET24a_acrB)	no									
Oxacillin			intermed. Effect (25% activity)	32x											
Ethidium			no effect	–											
Linezolid			no effect	–											
Novobiocin			100% inhibition	2x											
Rhodamine 6G			no effect	–											
SDS			no effect	–											
TPP			no effect	–											
Erythromycin	/	/	no effect	–	like wt (pET24a_acrB)	no									
Oxacillin			no effect	–											
Ethidium			intermed. Effect (25% activity)	8x											
Linezolid			no effect	–											
Novobiocin			intermed. Effect (25% activity)	4x											
Rhodamine 6G			100% inhibition	2x											
SDS			intermed. Effect (6% activity)	32x											
TPP			no effect	–											
Erythromycin	/	/	no effect	–	like wt (pET24a_acrB)	no									
Oxacillin			intermed. Effect (25% activity)	32x											
Ethidium			intermed. Effect (25% activity)	8x											
Linezolid			100% inhibition	–											
Novobiocin			intermed. Effect (25% activity)	4x											
F615A_F617A_R620A	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector?)	Erythromycin	/	/	intermed. effect (25% activity)	4x	like wt (pETHisAcrB)	yes apo: 4ZIV with ery: 4ZIO	27403665 + supplement						
G616	PC1 (sw-loop)			Erythromycin	intermed. Effect (25% activity)	32x	/	/	not determined	no	18936189				
				Clarithromycin	intermed. Effect (25% activity)	32x									
				Azithromycin	intermed. Effect (25% activity)	32x									
				Novobiocin	no effect	–									
				Levofloxacin	no effect	–									
				Minocycline	no effect	–									
				Chloramphenicol	no effect	–									
				Ethidium	no effect	–									
				Pyronin Y	no effect	–									
				Linezolid	no effect	–									
				Oxacillin	no effect	–									
				Rhodamine 6G								100% inhibition	2x		
				SDS								100% inhibition	–		
				TPP								100% inhibition	2x		
Erythromycin	/	/	100% inhibition	–	like wt (pET24a_acrB)	no									
Oxacillin			100% inhibition	2x											
Ethidium			100% inhibition	–											
Linezolid			100% inhibition	–											
Novobiocin			100% inhibition	–											
Rhodamine 6G			intermed. Effect (12.5% activity)	4x											
SDS			100% inhibition	2x											
TPP			intermed. Effect (12.5% activity)	8x											
Erythromycin	/	/	100% inhibition	2x	like wt (pET24a_acrB)	no									
Oxacillin			intermed. Effect (6% activity)	8x											
Ethidium			100% inhibition	2x											
Linezolid			100% inhibition	2x											
Novobiocin			100% inhibition	2x											
Growth Curves	Erythromycin (32mg/ml)	/	/	100% Inhibition (growth like neg. Ctrl. (=empty vector))	–	not determined	no	22121023							
Efflux transport	Doxorubicin	/	/	100% inhibition (no efflux of doxo, quenching of fluorescence (like neg. Ctrl = vector))	–	not determined									

			Growth Curves (neg. Ctrl. D407N mutant)	SDS	/	/	no effect (like wt)	-						
				Acriflavine	/	/	no effect (like wt)	-						
				Linezolid	/	/	no effect (like wt)	-						
				Chloramphenicol	/	/	no effect (like wt)	-						
				Berberine	/	/	no effect (like wt)	-						
				Ciprofloxacin	/	/	no effect (like wt)	-						
				Hoechst 33342	/	/	no effect (like wt)	-						
				Dicloxacillin	/	/	intermed. Effect (< wt; >D407N)	-						
				Oxacillin	/	/	no effect (like wt)	-						
				Novobiocin	/	/	no effect (like wt)	-						
				TPP	/	/	no effect (like wt)	-						
				Fusidic acid	/	/	intermed. Effect (< wt; >D407N)	-						
				Rifampicin	/	/	intermed. Effect (< wt; >D407N)	-						
				Azithromycin	/	/	intermed. Effect (< wt; >D407N)	-						
				Spiramycin	/	/	intermed. Effect (< wt; >D407N)	-						
				Bleomycin	/	/	no effect (like wt)	-						
G619	PC1 (sw-loop)	G619P	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Rhodamine 6G	/	/	no effect	-	like wt (pET24a_acrB)	no	28987732 + supplement			
				SDS	/	/	no effect	-						
				TPP	/	/	no effect	-						
				Erythromycin	/	/	no effect	-						
				Oxacillin	/	/	intermed. Effect (25% activity)	16x						
				Ethidium	/	/	intermed. Effect (25% activity)	8x						
				Linezolid	/	/	100% inhibition	2x						
				Novobiocin	/	/	100% inhibition	2x						
				Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	4x						
				SDS	/	/	100% inhibition	2x						
	G619P_G621P	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)			TPP	/	/	intermed. Effect (6% activity)	4x					
					Erythromycin	/	/	100% inhibition	-					
					Oxacillin	/	/	100% inhibition	2x					
					Ethidium	/	/	100% inhibition	2x					
					Linezolid	/	/	100% inhibition	-					
					Novobiocin	/	/	100% inhibition	-					
					Erythromycin	/	/	no effect	-					
					Novobiocin	/	/	no effect	-					
					Rhodamine 6G	/	/	no effect	-					
					SDS	/	/	no effect	-					
R620	PC1 (sw-loop)	R620C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl empty vector)	Erythromycin	/	/	no effect	-	not determined	no	24551234			
				Novobiocin	/	/	no effect	-						
G621	PC1 (sw-loop)	G621P	MIC (relative MIC values; neg. Ctrl = 1; pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Rhodamine 6G	/	/	no effect	-	like wt (pET24a_acrB)	no	28987732 + supplement			
				SDS	/	/	no effect	-						
				TPP	/	/	no effect	-						
				Erythromycin	/	/	no effect	-						
				Oxacillin	/	/	intermed. Effect (25% activity)	32x						
				Ethidium	/	/	intermed. Effect (25% activity)	8x						
				Linezolid	/	/	100% inhibition	2x						
				Novobiocin	/	/	100% inhibition	2x						
				Linezolid	no effect	-	/	/						
				G621S_K632R_Q846H	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)			Chloramphenicol				no effect	-	/
	Cefuroxime	no effect	-					/	/					
	Oxacillin	no effect	-					/	/					
	Clindamycin	no effect	-					/	/					
	Tetracycline	no effect	-					/	/					
	Tigecycline	no effect	-					/	/					
	Levofloxacin	no effect	-					/	/					
	Novobiocin	intermed. Effect (25% activity)	512x					/	/					
	Rifampicin	no effect	-					/	/					
	Clarithromycin	400% higher activity	-					/	/					
	N623	PC1 (sw-loop)	N623_Q624S	MIC (pos. Ctrl. AcrB wt; neg. ctrl. acrB:rpsL-neo - knockout)	Erythromycin	no effect	-	/	/	not determined	no	18936189		
Azithromycin					no effect	-	/	/						
Clarithromycin					no effect	-	/	/						
Novobiocin					no effect	-	/	/						
Levofloxacin					no effect	-	/	/						
Minocycline					no effect	-	/	/						
Chloramphenicol					no effect	-	/	/						
Ethidium					no effect	-	/	/						
Pyronin Y					no effect	-	/	/						
Linezolid					no effect	-	/	/						
		I626A	Efflux transport	Nitrocefin (conc.?)	/	/	no effect (Cp (at 175 μM C ₀) 20.3 μM (wt 19.5 μM))	-	not determined	no	27789287			
					/	/	no effect (K _m 2.5 ± 0.9 μM (wt 3.8 ± 1.0 μM))	-						
					/	/	no effect (v _{max} 0.033 ± 0.0003 nmol/s/mg (wt 0.039 ± 0.006 nmol/s/mg))	-						
				Ala-Naph (conc.?)	/	/	intermed. Effect (Cp (at 1 μM C ₀) 134 μM (wt 268 μM))	no negativ control						
					/	/	intermed. Effect (K _m 255 ± 77 μM (wt 176 ± 19 μM))	no negativ control						
					/	/	intermed. Effect (v _{max} 268 ± 44 nmol/s/mg (wt 82 ± 16 nmol/s/mg))	no negativ control						
					/	/	intermed. Effect (hill-coeff. 1.5 ± 0.1 (wt 1.7 ± 0.1))	no negativ control						
				Erythromycin	no effect	-	/	/						
				Clarithromycin	no effect	-	/	/						

I626	PC1	I626M	MIC (pos. Ctrl. AcrB wt; neg. ctrl. acrB:rpstl-neo - knockout)	Azithromycin	no effect	—	/	/	not determined	no	18936189		
				Novobiocin	no effect	—	/	/					
				Levofloxacin	no effect	—	/	/					
				Minocycline	no effect	—	/	/					
				Chloramphenicol	no effect	—	/	/					
				Ethidium	no effect	—	/	/					
				Pyronin Y	no effect	—	/	/					
				Linezolid	no effect	—	/	/					
				Oxacillin	no effect	—	/	/					
		Azteonam	/	/	800% higher activity	—							
		Carbencillin	/	/	400% higher activity	—							
		Sulbencillin	/	/	1600% higher activity	—							
		Oxacillin	/	/	no effect	—							
		Cloxacillin	/	/	intermed. Effect (25% activity)	256x							
		Nafcillin	/	/	no effect	—							
		Cefamandole	/	/	no effect	—							
		Novobiocin	/	/	no effect	—							
		Erythromycin	/	/	intermed. Effect (25% activity)	8x							
		tetracycline	/	/	no effect	—							
		Minocycline	/	/	intermed. Effect (25% activity)	4x							
		Nalidixic acid	/	/	no effect	—							
		Norflaxacin	/	/	no effect	—							
		Enoxacin	/	/	no effect	—							
		Trimethoprim	/	/	no effect	—							
		Triclosan	/	/	no effect	—							
		Acriflavine	/	/	intermed. Effect (12.5% activity)	16x							
		Crystal violet	/	/	no effect	—							
		Ethidium	/	/	intermed. Effect (12.5% activity)	16x							
		Rhodamine 6G	/	/	intermed. Effect (25% activity)	256x							
		TPP	/	/	intermed. Effect (25% activity)	128x							
		Benzalkonium	/	/	no effect	—							
		SDS	/	/	no effect	—							
		Deoxycholate	/	/	no effect	—							
I626R	MIC (relative MIC values; neg. ctrl = 1; pos. Ctrl. native AcrAB, neg. Ctrl. Strain ΔacrD and strain has no acrB)	Azteonam	—	—	1600% higher activity	—			like wt (pTHacrAB-His)	no	24558035		
		Carbencillin	/	/	1600% higher activity	—							
		Sulbencillin	/	/	1600% higher activity	—							
		Oxacillin	/	/	no effect	—							
		Cloxacillin	/	/	no effect	—							
		Nafcillin	/	/	no effect	—							
		Cefamandole	/	/	no effect	—							
		Novobiocin	/	/	no effect	—							
		Erythromycin	/	/	intermed. Effect (12.5% activity)	4x							
		Tetracycline	/	/	no effect	—							
		Minocycline	/	/	no effect	—							
		Nalidixic acid	/	/	no effect	—							
		Norflaxacin	/	/	100% inhibition	2x							
		Enoxacin	/	/	100% inhibition	2x							
		Trimethoprim	/	/	no effect	—							
		Triclosan	/	/	no effect	—							
		Acriflavine	/	/	intermed. Effect (12.5% activity)	16x							
		Crystal violet	/	/	no effect	—							
		Ethidium	/	/	intermed. Effect (6% activity)	8x							
		Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	128x							
		TPP	/	/	intermed. Effect (12.5% activity)	64x							
Benzalkonium	/	/	no effect	—									
SDS	/	/	no effect	—									
Deoxycholate	/	/	no effect	—									
I626R_E673G	MIC (relative MIC values; neg. ctrl = 1; pos. Ctrl. native AcrAB, neg. Ctrl. Strain ΔacrD and strain has no acrB)	Azteonam	—	—	1600% higher activity	—			like wt (pTHacrAB-His)	no	24558035		
		Carbencillin	/	/	1600% higher activity	—							
		Sulbencillin	/	/	1600% higher activity	—							
		Oxacillin	/	/	no effect	—							
		Cloxacillin	/	/	no effect	—							
		Nafcillin	/	/	no effect	—							
		Cefamandole	/	/	no effect	—							
		Novobiocin	/	/	no effect	—							
		Erythromycin	/	/	intermed. Effect (12.5% activity)	4x							
		Tetracycline	/	/	no effect	—							
		Minocycline	/	/	no effect	—							
		Nalidixic acid	/	/	no effect	—							
Norflaxacin	/	/	100% inhibition	2x									
Enoxacin	/	/	100% inhibition	2x									
Trimethoprim	/	/	no effect	—									
Triclosan	/	/	no effect	—									
Acriflavine	/	/	intermed. Effect (12.5% activity)	16x									
Crystal violet	/	/	no effect	—									
Ethidium	/	/	intermed. Effect (6% activity)	8x									
Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	128x									
TPP	/	/	intermed. Effect (12.5% activity)	64x									
Benzalkonium	/	/	no effect	—									
SDS	/	/	no effect	—									
Deoxycholate	/	/	no effect	—									
F628	PC1	Growth Curves	Erythromycin (32mg/ml)	/	/	growth like neg. Ctrl. (=empty vector)	—		not determined	no	22121023		
			Efflux transport	Doxorubicin	/	/	intermed. Effect (efflux of doxo, quenching of fluorescence (~50% of wt))	—		not determined		no	
		Efflux transport	Nitrocefin (conc.?)	/	/	no effect (Cp (at 175 μM C ₀) 22.0 μM (wt 19.5μM))	—			not determined	no	27789287	
				/	/	no effect (K _{0.5} 5.5 ± 2.9 μM (wt 3.8 ± 1.0 μM))	—						
				/	/	no effect (v _{max} 0.036 ± 0.0007 nmol/s/mg (wt 0.039 ± 0.006 nmol/s/mg))	—						
				/	/	no effect (Cp (at 1 μM C ₀) 287 μM (wt 268 μM))	—						
				/	/	no effect (K _{0.5} 145,143 μM (wt 176 ± 19 μM))	—						
		Efflux transport (determ. of EHT=efflux half times over 400s)	Ala-Naph (conc.?)	/	/	no effect (v _{max} 73,74 nmol/s/mg (wt 82 ± 16 nmol/s/mg))	—			not determined	no	21698261	
				/	/	no effect (hill-coeff. 1.9,2.0 (wt 1.7 ± 0.1))	—						
				1,2'-DNA: 0.25 μM	/	/	intermed. Effect (54 ± 12.9 s (wt: 20 ± 5 s))	no negativ control					
				1,2'-DNA: 1 μM	/	/	intermed. Effect (52 ± 13.1 s (wt: 19 ± 1.5 s))	no negativ control					
				1,2'-DNA: 4 μM	/	/	intermed. Effect (53 ± 13.1 s (wt: 24 ± 2.5 s))	no negativ control					
		1,2'-DNA: 16 μM	/	/	intermed. Effect (55 ± 11.9 s (wt: 27 ± 2.9 s))	no negativ control							
		1,2'-DNA: 64 μM	/	/	intermed. Effect (68 ± 14.6 s (wt: 37 ± 5.2 s))	no negativ control							
		MIC (pos. Ctrl. AcrB wt;	Oxacillin	no effect	—	/	/						
Novobiocin	intermed. Effect (25% activity)		32x	/	/								
Clarithromycin	intermed. Effect (12.5% activity)		16x	/	/								

		F628A	neg. ctrl. acrB:rpsL-neo - knockout)	Azithromycin	intermed. Effect (6% activity)	32x	/	/	like wt	no	18849422
				Pyronin Y	intermed. Effect (25% activity)	16x	/	/			
		F628C AcrB CL (=Cys-less, replaced by ??)	Efflux transport	Ethidium (2.5 µM)	100% inhibition (like neg. Ctrl)	—	/	/	not determined	no	20804453
				PABN (200 µM)	100% inhibition (like neg. Ctrl)	—	/	/			
F664	PC1	F664C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Epty vector)	Erythromycin	/	/	intermed. Effect (25% activity)	40x	not determined	no	24551234
				Novobiocin	/	/	intermed. Effect (25% activity)	80x			
F666	PC1	F666W	Growth Curves	Erythromycin (32mg/ml)	/	/	100% inhibition (growth like neg. Ctrl. (=empty vector))	—	not determined	no	22121023
				Efflux transport	Doxorubicin	/	/	intermed. Effect (efflux of doxo, quenching of fluorescence (~50% of wt))			
		F666C	MIC (pos. Ctrl AcrB; neg. Ctrl. Empty vector))	Erythromycin	/	/	intermed. Effect (25% activity)	80x	not determined	no	24551234
				Novobiocin	/	/	100% inhibition	2x	not determined	no	
		F666C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholate	/	/	no effect (70% activity)	—	not determined	no	17905989
				Cholate	/	/	100% inhibition	—	not determined	no	
				Cholate	/	/	intermed. Effect (15% activity)	—	not determined	no	
				Cholate	/	/	no effect (90% activity)	—	not determined	no	
				Cholate	/	/	no effect (65% activity)	—	not determined	no	
				Cholate	/	/	no effect (75% activity)	—	not determined	no	
L668	PC1	L668C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	—	not determined	no	24551234
				Novobiocin	/	/	no effect	—			
I671	PC1	I671T	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Linezolid	intermed. Effect (6% activity)	4x	/	/	not determined	no	27161641 + supplement
				Chloramphenicol	100% inhibition	—	/	/			
				Cefuroxime	intermed. Effect (6% activity)	4x	/	/			
				Oxacillin	intermed. Effect (25% activity)	4x	/	/			
				Clindamycin	intermed. Effect (25% activity)	32x	/	/			
				Tetracycline	100% inhibition	—	/	/			
				Tigecycline	intermed. Effect (25% activity)	4x	/	/			
				Levofloxacin	intermed. Effect (25% activity)	4x	/	/			
				Novobiocin	intermed. Effect (25% activity)	128x	/	/			
				Rifampicin	no effect	—	/	/			
				Clarithromycin	400% higher activity	—	/	/			
				Erythromycin	no effect	—	/	/			
				Azithromycin	no effect	—	/	/			
				Ethidium	—	intermed. Effect (44.6 ± 8)	/	/			
				Hoechst 33342	—	intermed. Effect (55.7 ± 7)	/	/			
				Berberine	—	Intermed. Effect (27.2 ± 7)	/	/			
				Pyronin Y	—	intermed. Effect (73.1 ± 5)	/	/			
				I671T_R699L_S807P	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Linezolid	intermed. Effect (6% activity)	4x			
		Chloramphenicol	100% inhibition			—	/	/			
		Cefuroxime	intermed. Effect (6% activity)			4x	/	/			
		Oxacillin	intermed. Effect (12.5% activity)			128x	/	/			
		Clindamycin	intermed. Effect (25% activity)			32x	/	/			
		Tetracycline	100% inhibition			2x	/	/			
		Tigecycline	no effect			—	/	/			
		Levofloxacin	intermed. Effect (12.5% activity)			4x	/	/			
		Novobiocin	no effect			—	/	/			
		Rifampicin	no effect			—	/	/			
		Clarithromycin	400% higher activity			—	/	/			
		Erythromycin	no effect			—	/	/			
		Azithromycin	no effect	—	/	/					
I671T_S813P_A840T	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Linezolid	intermed. Effect (6% activity)	4x	/	/	not determined	no			
		Chloramphenicol	100% inhibition	—	/	/					
		Cefuroxime	intermed. Effect (6% activity)	4x	/	/					
		Oxacillin	intermed. Effect (25% activity)	256x	/	/					
		Clindamycin	intermed. Effect (25% activity)	32x	/	/					
		Tetracycline	100% inhibition	—	/	/					
		Tigecycline	inhibition (25% activity)	2x	/	/					
		Levofloxacin	intermed. Effect (12.5% activity)	4x	/	/					
		Novobiocin	intermed. Effect (25% activity)	512x	/	/					
		Rifampicin	no effect	—	/	/					
Clarithromycin	400% higher activity	—	/	/							
Erythromycin	no effect	—	/	/							
Azithromycin	no effect	—	/	/							
				Linezolid	no effect	—	/	/			
				Chloramphenicol	no effect	—	/	/			
				Cefuroxime	no effect	—	/	/			
				Oxacillin	no effect	—	/	/			

V672	PC1	V672M	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Clindamycin	no effect	—	/	/	not determined	no	27161641 + supplement			
				Tetracycline	no effect	—	/	/						
				Tigecycline	no effect	—	/	/						
				Levofloxacin	no effect	—	/	/						
				novobiocin	no effect	—	/	/						
				Rifampicin	no effect	—	/	/						
				Clarithromycin	400% higher activity	—	/	/						
				Erythromycin	no effect	—	/	/						
				Azithromycin	no effect	—	/	/						
				Azithromycin	no effect	—	/	/						
E673	PC1	E673G	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Linezolid	/	/	no effect	—	not determined	no	27161641 + supplement			
				Chloramphenicol	/	/	no effect	—						
				Cefuroxime	/	/	no effect	—						
				Oxacillin	/	/	no effect	—						
				Clindamycin	/	/	no effect	—						
				Tetracycline	/	/	no effect	—						
				Tigecycline	/	/	no effect	—						
				Levofloxacin	/	/	no effect	—						
				Novobiocin	/	/	no effect	—						
				Rifampicin	/	/	no effect	—						
				Clarithromycin	/	/	no effect	—						
				Erythromycin	/	/	no effect	—						
				Azithromycin	/	/	no effect	—						
				Azithromycin	/	/	no effect	—						
				E673C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Erythromycin	/	/				no effect	—	not determined
		E673C AcrB CL (=Cys-less, replaced by ??)	Efflux transport	Nile Red + pyrene-malmeide (30 μM)	/	/	intermed. Effect (~ 50% blockage of transport of Nile Red compared to pos. Ctrl. AcrB CL)	—				not determined	no	2084453
		E673C AcrB CL (=Cys-less, replaced by ??)	Efflux transport	Nile Red + CPM (12 μM)	/	/	100% Inhibition (blockage of transport of Nile Red compared to pos. Ctrl. AcrB CL)	—				not determined	no	2084453
		E673G	MIC (pos. Ctrl. native AcrAB, neg. Ctrl. Strain ΔacrD and strain has no acrB)	Azteonam	/	/	no effect	—				like wt (pTHacrAB-His)	no	24558035
		Carbenicillin	/	/	no effect	—								
		Sulbenicillin	/	/	no effect	—								
		Oxacillin	/	/	no effect	—								
		Cloxacillin	/	/	no effect	—								
		Nafcillin	/	/	no effect	—								
		Cefamandole	/	/	no effect	—								
		Novobiocin	/	/	no effect	—								
		Erythromycin	/	/	no effect	—								
		Tetracycline	/	/	no effect	—								
		Minocycline	/	/	no effect	—								
		Nalidixic acid	/	/	no effect	—								
		Norfloxacin	/	/	no effect	—								
		Enoxacin	/	/	no effect	—								
		Trimethoprim	/	/	no effect	—								
		Triclosan	/	/	no effect	—								
		Acriflavine	/	/	no effect	—								
		Crystal violet	/	/	no effect	—								
Ethidium	/	/	no effect	—										
Rhodamine 6G	/	/	no effect	—										
TPP	/	/	no effect	—										
Benzalkonium	/	/	no effect	—										
SDS	/	/	no effect	—										
Deoxycholate	/	/	no effect	—										
Erythromycin	/	/	no effect	—										
Novo	/	/	no effect	—										
T676	PC1	T676C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	—	not determined	no	24551234			
T676C AcrB CL (=Cys-less, replaced by ??)	Efflux transport	Nile Red + pyrene-malmeide (30 μM)	/	/	intermed. Effect (~ 50% blockage of transport of Nile Red compared to pos. Ctrl. AcrB CL)	—	not determined	no	2084453					
A677	PC1	A677W	Growth Curves	Erythromycin (32mg/ml)	/	/	100% Inhibition (growth like neg. Ctrl. (=empty vector))	—	not determined	no	22121023			
A677W	Efflux transport	Doxorubicin	/	/	intermed. Effect (efflux of doxo, quenching of fluorescence (~50% of wt))	—	not determined	no	17905989					
T678C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt)	Cholate	/	/	no effect (80% activity)	—	not determined	no	17905989					
T678S	Efflux transport (determ. of relative efflux rates; pos. Ctrl. (AcrB) = 1.00)	n-octane	/	/	1.07	—	not determined	no	23693002					
T678A	n-octane	/	/	1.26	—	not determined	no	23693002						
T678A	a-pinene	/	/	3.20	—	not determined	no	23693002						
D681	Loop PC1-PC2	D681W	Efflux transport (Compet.)	1. Doxorubicin; 2. Erythromycin	/	/	better than wt (still doxo export; no effect for ery)	—	not determined	no	22121023			
A688	Loop PC1-PC2	Q688C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt)	Cholate	/	/	no effect (90% activity)	—	not determined	no	17905989			
Q697	Loop PC1-PC2	Q697C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt)	Cholate	/	/	no effect (90% activity)	—	not determined	no	17905989			
S715	PC2	S715C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	—	was expressed (no pos. Ctrl on gel)	no	11754746			
R717C	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	—	not determined	no	24551234					
R717C	Novobiocin	/	/	no effect	—	not determined	no	24551234						
R717C	Linezolid	/	/	no effect	—	not determined	no	24551234						
R717C	Chloramphenicol	/	/	no effect	—	not determined	no	24551234						
R717C	Cefuroxime	/	/	no effect	—	not determined	no	24551234						
R717C	Oxaci	/	/	no effect	—	not determined	no	24551234						
R717C	Clindamycin	/	/	no effect	—	not determined	no	24551234						

R717	PC2	R717S	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Tetracycline	/	/	no effect	—	not determined	no	27161641 + supplement				
				Tigecycline	/	/	no effect	—							
				Levofloxacin	/	/	no effect	—							
				Novobiocin	/	/	no effect	—							
				Rifampicin	/	/	no effect	—							
				Clarithromycin	/	/	400% higher activity	—							
				Erythromycin	/	/	no effect	—							
				Azithromycin	/	/	no effect	—							
Q726	DC	Q726CAcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL; neg. Ctrl. Empty vector)	Rhodamine 6G	/	/	no effect	—	not determined	no	18223659				
				Berberine	/	/	no effect	—							
				Erythromycin	/	/	no effect	—							
				Oxacillin	/	/	no effect	—							
		L578C_Δ627C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL, neg. control empty vector)	Erythromycin	/	/	no effect	—							
				Novobiocin	/	/	no effect	—							
				TPP	/	/	no effect	—							
				Rhodamine 6G	/	/	no effect	—							
K728	DC	K728C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL; neg. Ctrl. Empty vector)	Berberine	/	/	no effect	—	not determined	no	18223659				
				Erythromycin	/	/	no effect	—							
				Oxacillin	/	/	no effect	—							
				n-octane	/	/	1.51	—							
				α-pinene	/	/	3.89	—							
				Acriflavine	/	/	no effect	—							
				Erythromycin	/	/	no effect	—							
				Novobiocin	/	/	no effect	—							
M774	DC	M774K AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	Rhodamine 6G	/	/	no effect	—	not determined	no	24007302				
				TPP	/	/	no effect	—							
				Acriflavine	/	/	no effect	—							
				Erythromycin	/	/	no effect	—							
		M774R AcrB CL (=Cys-less, replaced by A)		Novobiocin	/	/	no effect	—	not determined	no					
				Rhodamine 6G	/	/	no effect	—							
				TPP	/	/	no effect	—							
				Acriflavine	/	/	no effect	—							
		M774R_Δ780A AcrB CL (=Cys-less, replaced by A)		Erythromycin	/	/	no effect	—	not determined	no					
				Novobiocin	/	/	no effect	—							
				Rhodamine 6G	/	/	no effect	—							
				TPP	/	/	no effect	—							
		M774K_Δ78A AcrB CL (=Cys-less, replaced by A)		Acriflavine	/	/	no effect	—	not determined	no					
				Erythromycin	/	/	no effect	—							
				Novobiocin	/	/	no effect	—							
				Rhodamine 6G	/	/	no effect	—							
A777	DC	A777C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL; neg. Ctrl. Empty vector)	TPP	/	/	no effect	—	not determined	no	18223659				
				Rhodamine 6G	/	/	no effect	—							
				Berberine	/	/	no effect	—							
				Erythromycin	/	/	no effect	—							
		MIC (pos. Ctrl. AcrB CL; neg. Ctrl. D407A mutant)		Oxacillin	/	/	no effect	—							
				Erythromycin	/	/	no effect	—							
				Novobiocin	/	/	no effect	—							
				Rhodamine 6G	/	/	no effect	—							
R780	DC	R780A	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	no effect	—	like wt (pQE70-AcrB)	no (but near UV CD spectra; similar to wt)	24007302				
				Acriflavine	/	/	100% inhibition	—							
				Erythromycin	/	/	100% inhibition	—							
				Novobiocin	/	/	100% inhibition	—							
		R780K		Rhodamine 6G	/	/	100% inhibition	—	like wt (pQE70-AcrB)	no					
				TPP	/	/	100% inhibition	—							
				Acriflavine	/	/	100% inhibition	2x							
				Erythromycin	/	/	intermed. Effect (25% activity)	8x							
		R780M		Novobiocin	/	/	intermed. Effect (25% activity)	8x	like wt (pQE70-AcrB)	no					
				Rhodamine 6G	/	/	intermed. Effect (12.5% activity)	8x							
				TPP	/	/	intermed. Effect (6.25% activity)	4x							
				Acriflavine	/	/	100% inhibition	—							
		R780Q		Erythromycin	/	/	100% inhibition	—	like wt (pQE70-AcrB)	no					
				Novobiocin	/	/	100% inhibition	—							
				Rhodamine 6G	/	/	100% inhibition	—							
				TPP	/	/	100% inhibition	—							
		L822		PC2	L822P	Plate Dilution Assay	1-hexene saturated atmosphere	/	/	10 ⁹ cells: better than wt		—	not determined	no	25450012
								/	/	10 ⁴ cells: better than wt		—			
							Linezolid	no effect	—	/		/			
							Chloramphenicol	no effect	—	/		/			
				Cefuroxime	no effect	—	/	/							
				Oxacillin	no effect	—	/	/							
				Clindamycin	no effect	—	/	/							
				Tetracycline	no effect	—	/	/							

L828	PC2	L828F	MIC (pos. Ctrl. acrB (wt) from genome; neg. Ctrl. ΔacrB)	Tigecycline	no effect	—	/	/	not determined	no	27161641 + supplement	
				Levofloxacin	no effect	—	/	/				
				Novobiocin	no effect	—	/	/				
				Rifampicin	no effect	—	/	/				
				Clarithromycin	400% higher activity	—	/	/				
				Erythromycin	no effect	—	/	/				
				Azithromycin	no effect	—	/	/				
				Linezolid	no effect	—	/	/				
				Chloramphenicol	intermed. Effect (25% activity)	4x	/	/				
				Cefuroxime	no effect	—	/	/				
		Oxacillin	no effect	—	/	/						
		Clindamycin	no effect	—	/	/						
		Tetracycline	no effect	—	/	/						
		Tigecycline	no effect	—	/	/						
		Levofloxacin	no effect	—	/	/						
		Novobiocin	no effect	—	/	/						
		Rifampicin	no effect	—	/	/						
		Clarithromycin	400% higher activity	—	/	/						
		Erythromycin	no effect	—	/	/						
		Azithromycin	no effect	—	/	/						
Q830	PC1	Q830C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt)	Cholate	/	/	no effect (90% activity)	—	not determined	no	17905989	
S836	PC2	5C AcrB CL (=Cys-less, replaced by S)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	100% inhibition	—	less expressed (compared to other mutants)	no	11754746	
T837	PC2	T837C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL; neg. Ctrl. Empty vector)	TPP	/	/	no effect	—	not determined	no	18223659	
				Rhodamine 6G	/	/	no effect	—				
				Berberine	/	/	no effect	—				
				Erythromycin	/	/	no effect	—				
				Oxacillin	/	/	no effect	—				
E839	PC2	E839C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB CL; neg. Ctrl. Empty vector)	TPP	/	/	no effect	—	not determined	no	18223659	
				Rhodamine 6G	/	/	no effect	—				
				Berberine	/	/	no effect	—				
				Erythromycin	/	/	no effect	—				
				Oxacillin	/	/	no effect	—				
M844	PC2	M844L	Efflux transport (determ. of relative efflux rates; pos. Ctrl. (AcrB) = 1.00)	n-octane	/	/	1.15	—	not determined	no	23693002	
		a-pinene		/	/	1.31	—					
		n-octane		/	/	2.41	—					
		a-pinene		/	/	3.00	—					
G854	PC2	G854C AcrB CL (=Cys-less, replaced by S)	MIC (calculated relative activity to wt)	Cholate	/	/	no effect (90% activity)	—	not determined	no	17905989	
S863	Hoisting Loop	S863C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	—	was expressed (no pos. Ctrl on gel)	no	11754746	
L868	Hoisting Loop	L868P	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. Knockout acrB)	Cloxacillin	/	/	no effect	—	not determined	no	29118749	
				Benzalkonium	/	/	intermed. Effect (25% activity)	32x				
				Ethidium	/	/	no effect	—				
				Crystal violet	/	/	no effect	—				
				Novobiocin	/	/	no effect	—				
				Rhodamine 6G	/	/	no effect	—				
				Minocycline	/	/	no effect	—				
				Acriflavin	/	/	no effect	—				
				Erythromycin	/	/	no effect	—				
				Cloxacillin	/	/	no effect	—				
		L868P_Q872P	Benzalkonium	/	/	no effect	—					
			Ethidium	/	/	no effect	—					
			Crystal violet	/	/	no effect	—					
			Novobiocin	/	/	no effect	—					
			Rhodamine 6G	/	/	no effect	—					
			Minocycline	/	/	no effect	—					
			Acriflavin	/	/	no effect	—					
			Erythromycin	/	/	no effect	—					
			Erythromycin	/	/	no effect	—					
			Novobiocin	/	/	no effect	—					
L886G	TM8	L886G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Rhodamine 6G	/	/	no effect	—	like wt (pQE70_acrB)	no	24854514	
				TPP	/	/	no effect	—				
				Efflux transport	Ethidium (5 μM)	/	/	100% Inhibition (E1Br accumulation rate (slop, s ⁻¹) higher like wt)				—
				Erythromycin	/	/	no effect	—				
L886G_E893G	L886G_E893G	L886G_E893G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Novobiocin	/	/	intermed. Effect (25% activity)	16x	like wt (pQE70_acrB)	no		
				Rhodamine 6G	/	/	no effect	—				
				TPP	/	/	intermed. Effect (6% activity)	16x				
				Efflux transport	Ethidium (5 μM)	/	/	100% Inhibition (E1Br accumulation rate (slop, s ⁻¹) higher like wt)			—	
L886G_E893G_W895G	L886G_E893G_W895G	L886G_E893G_W895G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Erythromycin	/	/	intermed. Effect (25% activity)	16x	like wt (pQE70_acrB)	no		
				Novobiocin	/	/	100% inhibition	2x				
				Rhodamine 6G	/	/	no effect	—				
				TPP	/	/	intermed. Effect (3% activity)	4x				

			Efflux transport	Ethidium (5 µM)	/	/	100% inhibition (EtBr accumulation rate (slop, s ⁻¹) higher like wt)	–			
Y892	TM8	2C AcrB CL (=Cys-less, replaced b	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
E893	TM8	E893G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	–	for times higher like wt (pQE70_acrB)	no	24854514
				Novobiocin	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				TPP	/	/	no effect	–			
		Efflux transport	Ethidium (5 µM)	/	/	no effect (EtBr accumulation rate (slop, s ⁻¹) like wt)	–				
		E893G_W895G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	–	for times higher like wt (pQE70_acrB)	no	
				Novobiocin	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
TPP	/			/	no effect	–					
Efflux transport	Ethidium (5 µM)	/	/	100% inhibition (EtBr accumulation rate (slop, s ⁻¹) higher like wt)	–						
S894	Loop TM8-9	S894C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
W895	Loop TM8-9	W895G	MIC (pos. Ctrl. AcrB wt, neg. Ctrl. Empty vector)	Erythromycin	/	/	no effect	–	not determined	no	24854514
				Novobiocin	/	/	no effect	–			
				Rhodamine 6G	/	/	no effect	–			
				TPP	/	/	no effect	–			
			Efflux transport	Ethidium (5 µM)	/	/	100% inhibition (EtBr accumulation rate (slop, s ⁻¹) higher like wt)	–			
L921	Loop TM9-10	L921C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	100% inhibition	–	was expressed (no pos. Ctrl on gel)	no	11754746
N923	Loop TM9-10	3C AcrB CL (=Cys-less, replaced b	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
		N923P	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Ciprofloxacin (5 ng/ml)	/	/	no effect (behavior like wt)	–	like wt (pET21a_acrB)	no	28497315
V925	Loop TM9-10	V925P	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Ciprofloxacin (5 ng/ml)	/	/	no effect (behavior like wt)	–	like wt (pET21a_acrB)	no	28497315
Y926	TM10	5C AcrB CL (=Cys-less, replaced b	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	less expressed (compared to other mutants)	no	11754746
Q927	TM10	L927S	Plate Dilution Assay	1-hexene saturated atmosphere	/	/	10 ⁹ cells: better than wt 10 ⁷ cells: better than wt	–	not determined	no	25450012
T933	TM10	T933A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt	no	17015667
				TPP	/	/	no effect	–	not determined		
			MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Oxacillin	/	/	no effect	–	not determined		
				Rhodamine 6G	/	/	no effect	–	not determined		
				Berberine	/	/	no effect	–	not determined		
				Erythromycin	/	/	no effect	–	not determined		
				Linezolid	/	/	no effect	–	not determined		
T933V	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Ciprofloxacin (5 ng/ml)	/	/	no effect (behavior like wt)	–	ke wt (pET21a_acr	no	28497315		
S938	TM10	S938A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrB _{H10})	no	17015667
K940	TM10	K940A	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. Ctrl. D407N)	Ciprofloxacin (5 ng/ml)	/	/	100% inhibition	–	ke wt (pET21a_acr	no	28497315
				MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	100% inhibition	–	like wt (pSAcrB _{H10})	yes PDB ID: 2HQF (3.38Å) PMID: 17015668
			MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	100% inhibition	–	not determined		
				Oxacillin	/	/	100% inhibition	–			
				Rhodamine 6G	/	/	100% inhibition	–			
				Berberine	/	/	100% inhibition	–			
				Erythromycin	/	/	100% inhibition	–			
				Linezolid	/	/	100% inhibition	–			
				TPP	/	/	no effect	–			
				Oxacillin	/	/	no effect	–			
		Rhodamine 6G	/	/	no effect	–					
		Berberine	/	/	no effect	–					
		Erythromycin	/	/	no effect	–					
		Linezolid	/	/	no effect	–					
		K940R	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	intermed. Effect (6% activity)	4x	not determined		
				Oxacillin	/	/	intermed. Effect (12.5% activity)	4x			
				Rhodamine 6G	/	/	intermed. Effect (25% activity)	4x			
				Berberine	/	/	100% inhibition	2x			
				Erythromycin	/	/	100% inhibition	2x			
				Linezolid	/	/	100% inhibition	–			
TPP	/			/	100% inhibition	–					
K940H	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)			TPP	/	/	100% inhibition	–		no	
				Oxacillin	/	/	100% inhibition	–			
				Rhodamine 6G	/	/	100% inhibition	–			
		Berberine	/	/	100% inhibition	–					
		Erythromycin	/	/	100% inhibition	–					
		Linezolid	/	/	100% inhibition	–					
		TPP	/	/	100% inhibition	–					

		K940M		Oxacillin	/	/	100% inhibition	–					
				Rhodamine 6G	/	/	100% inhibition	–				no	
				Berberine	/	/	100% inhibition	–					
				Erythromycin	/	/	100% inhibition	–					
				Linezolid	/	/	100% inhibition	–					
N941	TM10	N941A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	ke wt (pET21a_crf)	no		17015667	
			MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	no effect	–			not determined	no	1942558
				Oxacillin	/	/	no effect	–					
				Rhodamine 6G	/	/	no effect	–					
				Berberine	/	/	no effect	–					
				Erythromycin	/	/	no effect	–					
L944A	Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. ctrl. D407N mutant)	Ciprofloxacin (5 ng/mL)	/	/	intermed. Effect (less growth)	–	ke wt (pET21a_crf)	no		28497315			
L944N			/	/	100% inhibition	–	ke wt (pET21a_crf)	no					
L944T			/	/	intermed. Effect (less growth)	–	ke wt (pET21a_crf)	no					
A949	TM10	A949C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no		11754746	
A963	TM10	A963C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no		11754746	
R971	TM11	R971A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	100% inhibition	–	like wt (pAcrBHis)			17015667	
			MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	100% inhibition	–	not determined				19425588
				Oxacillin	/	/	100% inhibition	–	not determined				
				Rhodamine 6G	/	/	100% inhibition	–	not determined				
				Berberine	/	/	100% inhibition	–	not determined				
				Erythromycin	/	/	100% inhibition	–	not determined				
				Linezolid	/	/	100% inhibition	–	not determined				
Plate Dilution Assay (pos. Ctrl. AcrB wt; neg. ctrl. D407N)	Ciprofloxacin (5 ng/mL)	/	/	100% inhibition	–	ke wt (pET21a_crf)	no		28497315				
R973	TM11	R973A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pAcrBHis)	no		17015667	
P974	TM11	P974A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pAcrBHis)	no		17015667	
M977	TM11	M977A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pAcrBHis)	no		17015667	
T978	TM11	M978T	Efflux transport (determ. of relative efflux rates; pos. Ctrl.)	n-octane	/	/	intermed. Effect (0.77)	–	not determined	no		2363002	
			a-pinene	/	/	intermed. Effect (0.78)	–						
		T978A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	intermed. Effect (10% activity)	–	like wt			17015667	
			MIC (pos. Ctrl. AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	100% inhibition	–	not determined				19425588
				Oxacillin	/	/	100% inhibition	–	not determined				
				Rhodamine 6G	/	/	intermed. Effect (25% activity)	8x	not determined				
				Berberine	/	/	100% inhibition	2x	not determined				
				Erythromycin	/	/	intermed. Effect (25% activity)	4x	not determined				
		Linezolid	/	/	100% inhibition	2x	not determined						
		T978G	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	intermed. effect (20% activity)	–	like wt	no		17015667	
		T978S			/	/	no effect (80% activity)	–	like wt	no			
		T978C			/	/	no effect (70% activity)	–	like wt	no			
		T978V			/	/	no effect (65% activity)	–	like wt	no			
		T978M			/	/	no effect (60% activity)	–	like wt	no			
		T978I			/	/	no effect (50% activity)	–	like wt	no			
		T978L			/	/	intermed. Effect (30% activity)	–	like wt	no			
		T978N			/	/	intermed. Effect (40% activity)	–	like wt	no			
T978F	/	/			intermed. Effect (10% activity)	–	like wt	no					
T978D	/	/			intermed. Effect (10% activity)	–	like wt	no					
T978K	/	/			intermed. Effect (5% activity)	–	like wt	no					
T978H	/	/			intermed. Effect (10% activity)	–	like wt	no					
T978V_P974A	/	/			no effect (65% activity)	–	like wt	no					
T978V_S979A	/	/			intermed. Effect (6% activity)	–	like wt	no					
T978S_S979A	/	/			intermed. Effect (7% activity)	–	like wt	no					
S979	TM11	S979A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect (70% activity)	–	like wt (pAcrBHis)	no		17015667	
T993	Loop TM11-12	T993A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pAcrBHis)	no		17015667	
S997	TM12	S997C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no		11754746	

Q1000	TM12	Q1000A	MIC (calculated relative activity to wt and under consideration of neg. Ctrl.)	Cholic acid	/	/	no effect	–	like wt (pSAcrBHis)	no	17015667
A1002	TM12	A1002C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
T1015	TM12	T1015A	MIC (calculated relative activity to wt)	Cholic acid	/	/	no effect	–	like wt (pSAcrBHis)	no	17015667
			MIC (pos. Ctrl AcrB wt; neg. Ctrl. empty vector)	TPP	/	/	no effect	–	not determined		
				Oxacillin	/	/	no effect	–	not determined		
				Rhodamine 6G	/	/	no effect	–	not determined		
				Berberine	/	/	no effect	–	not determined		
				Erythromycin	/	/	no effect	–	not determined		
				Linezolid	/	/	no effect	–	not determined		
V1028	after TM12	A1028M	Efflux transport (determ. of relative efflux rates; pos. Ctrl.	n-octane	/	/	no effect (0.91)	–	not determined	no	23693002
F1033	after TM12	F1033Y	Plate Dilution Assay	1-hexene saturated atmosphere	/	/	10 ⁸ cells: better than wt	–	not determined	no	25450012
					/	/	10 ⁸ cells: better than wt	–			
S1034	after TM12	S1034C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746
S1043	after TM12	S1043C AcrB CL (=Cys-less, replaced by A)	MIC (pos. Ctrl. AcrB wt; neg. Ctrl. N43 strain)	Novobiocin	/	/	no effect	–	was expressed (no pos. Ctrl on gel)	no	11754746