Supplementary Material

Structural and Phylogenetic Analyses of Resistance to Nextgeneration Aminoglycosides Conferred by AAC(2') Enzymes

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Figure S1: Panel of aminoglycosides used in crystallographic studies. Next-generation aminoglycoside features are colored in blue. Site of 2'-acetylation is colored in green, while 2'-hydroxyl of non-substrate, amikacin, is colored in red. Carbons are numbered as per their aminoglycoside ring, i.e., central ring, prime ring, or double-prime ring.

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Non-binders	Cellulosimicrobium cellulans	1	MТ		T	гτя	DA	SYT	LE	svi	DW	ADF	RA	A	٩L	RD	AN	1D	AE	MC	ÀAI	RY	٩S	٩GI	РМ	sĂ	AA	H,	٩A	LΤ	٧D	PA	A	56
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Likely binders	Vibrio cholerae	1				• • •	ML		LN	ND	EL				EL	IK		S	EA	Y.	E	- 1	- D			YY	FS			NA	К-		-	42
	Providencia stuartii [AAC(2')-la]	-¦	MG					YRS	ιĤ	TSC		TLS	SEK		ÂL			1	EG	F	E	3 -	- 6	SI	H -		FA	H		GG	м-		-	42
Binders	Mycobacterium tuberculosis [AAC(2')-lc]	_1	MH		T	۲Q۱	/HT/	ARL	VH	TAI	DL	DSE	TF	Q	DI	RQ	M٧	Ť	GA	F٠	Ā	G -	- D	ΤI	E -	ΤD	WE	н	T L (GG	M -		-	48
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Plasmid-encoded	Deinococcus wulumuqiensis Deinococcus sp. NW-56	1	MTR	PPG	КРА 	10/						PG				RF		. D		Ŷ.	F	1	Ľ	5	5-					GG	1:		-	53 48
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	Mycolicibacter senuensis	- 52														85 6 P	1			Y Y	12					RG		Ŀ			LD	AC		107
Unlikely binders	Klenkia soli	41	1.1	ŇĹ	AS-		RDG	ELI	GH	AA	vv	GRO		A	GG	RT	- 1	B	τĜ	Ϋ́		ĀV	AV	AA	AA	RR		Т	AG	A L	MA	EVI	Ě	96
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Non-binders	Cellulosimicrobium cellulans Mycolicibacter senuensis	109 108 104	RV/ QV DE	ARA IRG	AGA - AF - DY		RV I /GA		GD	RQI	PE	AEA SRF - VV			AL SR RL	GW GW GW	T - L P E L	w	RGRG	PI	IS F	V L	S P TE	TGI	PQ VL	RT PT	P P A	E I		lu1 GT	49 - V - V	FVI	- 1 M 1 L 1	40
Non-binders Unlikely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli	109 108 104 97	RV QV DE RL	ARA IRG IG-	AGA - AF - DY - GF		RV I /GA _AA _GA		GD AE SS SA	RQI	PE - 1 - D	AEA SRF - V\ GAA	ALY RIY /FY			GW GW GW	T - L P E L T F	W W	RGRGTG	PI	F	V L I R C L	SP TE TP		PQ VL VV	RT PT	- P P A	E		lu1 GT GS	49 - V - V WV	F VI M I I Y VI	- 1 M 1 L 1 L 1	40 62 54 49
Non-binders Unlikely binders Likely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli	109 108 104 97 99	RV QV DE RL TL	RA RG G - RG D -	AGA - AF - DY - GF - EF		RV I /GA _AA _GA _GA		GD AE SS SA	RQI	PE - 1 - 0	AEA SRF - VV GAA - HE				GW GW GW GW	T - L F E L T F S L	W	RGRGEG	P I P I P I	F		S P TE TP			RTPT	- P P A - C	E		lu1 GT GS	49 - V - V WV - V	F VI M I I Y VI M I Y	- 1 M 1 L 1 L 1 Y 1	40 62 54 49 49
Non-binders Unlikely binders Likely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkla soli Vibrio cholerae Acinetobacter baumannii	109 108 104 97 99 100	RV/ QV DE RL TL ETI	RA I RG I G - I RG I D - TH	AGA - AF - DY - GF - EF LEY		RV I /GA GA GA		GD	RQI	PE - I - D 	AEA SRF - V\ GAA - HE						W	RGREGE	PI PI PI	FRGS		S P TE TP DQ			RT PT FS	- P P A C P - D			GT GS	49 - V - V - V - V - V - I		- 1 L 1 L 1 Y 1	40 62 54 49 49 51
Non-binders Unlikely binders Likely binders Binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Providencia stuartii [AAC(2')-la] Providencia stuartii [AAC(2')-la]	109 108 104 97 99 100 102 105	RV QV DE RL TL ETI KI	RA RG RG D- TH AS	AGA - AF - DY - GF - EF LEY - CY		GA GA GA GA GA GA		GD AE SSA SE ED SA	RQI	PE - 1 - 0 - 0 - 0 - 0 - 0 - 0 - 0	AEA SRF - V\ GAA - HE - EA GQH							RGGGGGG		F G S F					RT PT FS LT RS	P P A C P - D - 1 - P			GS GG	49 - V - V - V - V - V - V - V		- 1 I 1 L 1 Y 1 V 1 L 1	40 62 54 49 51 56 59
Non-binders Unlikely binders Likely binders Binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycobacterium tuberculosis [AAC(2')-lc] Mycolacterium arabiense	109 108 104 97 99 100 102 105 82	RV/ QV DE RL TL ETI KI QVN	RA IRG IG- ID- TH IAS MRG /RG	AGA - AF - DY - GF - EF LEY - CY - AY		GA GA GA GA GA GA GA		GD AE SAE SAE SAE SAE SAE	RQI	PE - I - D - D - R - R	AEA SRF - V\ GAA - HEA GQF GRF							RGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		F					RT PT FS LT RS RT	P P A C P - D - D - D - D - D - T				49 - V - V - V - V - V - V - V - V - V	FVI MII MI MGV FVI FVI	- 1 L 1 L 1 Y 1 L 1	40 62 54 49 51 56 59 36
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded	Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycolicibacterium tuberculosis [AAC(2')-lc] Mycolicibacterium arabiense Deinococcus wilumuqiensis	109 108 104 97 99 100 102 105 82 110	RV QV DE RL EL K QV RQV	RG IG- ID- TH IAS /RG /AR	AGA - AF - DY - GF LEY - CY - AY - AY		GA GA GA GA GA GA GA		GDE SSAED SSAED SSAED SSAED SSAED	RQI	PE - 1 - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0	AEA SRF - V\ GAA - HE GQF GRF GLC		ASEDARE	AL SR RL SR AR SR AR				RG RG EG KG CG RG RG	PI PI NI KI PI	F G S F S S				PQ VV VV SY VV VV VV VV VV VV VV VV VV VV VV VV VV	RT PT FS LT RS RT PT	P P A C - D - D - I - P - T - P				49 - V - V - V - V - V - V - V - V - V - V	FVI MI ML FVI FVI	- M L L Y Y N L L Y	40 62 54 49 51 56 59 36 64
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkla soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycobacterium tuberculosis [AAC(2')-lc] Mycolicibacterium arabiense Deinococcus wulumugiensis Deinococcus sp. NW-56	109 108 104 97 99 100 102 105 82 110 105	RV QE L L L L L Q V V R L Q V	RA IRG ID- TH IAS /RG /AR /AR	AGA - AF - DY - GF - EF LEY - CY - AY - GY		GA GA GA GA GA GA GA GA		GDE SAE SAE SAE SAE SAE SAE SAE SAE SAE SA	RQI	PE - 1 - D - D - R - A - E - E	AEA SRF - VV GAA - HE GQF GLC GLC			AL SR RL SR AR SR AR	GW GW GW GW GW GW GW GW GW GW			RGGGGGGG		F G S F S S S S S S				PQL VVE VVE VVE VVE VVE VVE VVE VVE VVE VV	RT PT FS LT RS RT PT PT	P P A C - C - C - C - C - C - C - C - C -				49 - V - V - V - V - V - V - V - V - V - V		- 1 1 L 1 1 Y 1 L 1 1 Y 1 L 1 Y 1	140 62 54 49 51 56 59 36 64 59
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded	Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Providencia stuartii [AAC(2')-Ia] Providencia stuartii [AAC(2')-Ia] Mycobacterium tuberculosis [AAC(2')-Ic] Mycolicibacterium tuberculosis Deinococcus wilumuqiensis Deinococcus sp. NW-56	109 108 104 97 99 100 102 105 82 110 105	RV QUE RL EL KQV RQV RQV RQV	RA I RG I D- I AS I AS / RG / AR / AR	AGA - AF - DY - GF - EF - CY - AY - GY - GY		GA GA GA GA GA GA GA GA GA		GDESSAEDDAESS	RQI	PE - 1 - D - R - R - E	AEA SRF - V\ GAA - HE GQF GQL GQL			AL SRL SRL SRL SRL SRL SRL SRL SRL SRL SR	GW GW GW GW GW GW GW GW GW GW					F G S F S S S S S S S S S S S S S S S S					RT PT FS RT RS RT PT	P P A P - C - C - C - C - C - C - C - C - C - C				49 - V - V - V - V - V - V - V - V - V		- 111111111111111111111111111111111111	140 162 154 149 151 156 159 136 64 59
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded	Cellulosimicrobium cellulans <u>Mycolicibacter senuensis</u> Chioroflexus aggregans <u>Klenkia soli</u> Vibrio cholerae <u>Acinetobacter baumannii</u> Providencia stuartii [AAC(2')-la] <u>Mycoliciberium tuberculosis [AAC(2')-lc]</u> <u>Mycoliciberium tuberculosis</u> <u>Deinococcus wulumuqiensis</u> <u>Deinococcus sp. NW-56</u> Cellulosimicrobium cellulans	109 108 104 97 99 100 102 105 82 110 105	RV QDE RTL KQV RU RU RU RU	RA IRG ID- TH IAS /RG /AR /AR	AGA - AF - DY - GF - EF - EF - CY - AY - GY - GY		GA GA GA GA GA GA GA				PE - 1 - D - R - R - R - E - E	AE/ SRF - VV GA/ - HE GQF GGL GGL/ RCV			ALSRARL SRARL SRARL SRARL	GW GW GW GW GW GW GW GW GW GW GW					F R G S F S S S S S S S S S S S S S S S S S					RT PT FS LT RS RT PT	P P A C - C - C - C - C - C - C - C - C -				49 - V - V - V - V - V - V - V - V - V	FVI MI ML MGV FVI FVI	- 111111111111111111111111111111111111	140 162 154 149 151 156 159 136 164 59 64
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuarii (AAC(2')-la) Mycoloibacterium ruberculosis [AAC(2')-lc] Mycoloibacterium arbiense Deinococcus wulumuqiensis Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycoloibacter senuensis	109 108 104 97 99 100 102 105 82 110 105 82 110 105	RV/ QV DE RL TL ETI KI QV QV RQV RQV RQV RQV	RG IG- IRG ID- IAS /RG /AR /AR	AGA - A F - DY - G F - E F L E Y - C Y - A Y - G Y - G Y		GA GA GA GA GA GA GA GA GA GA				PE - 1 D D R - A - E - E	AEA SRF - VV GAA - HE GQA ARF GQC GL/ RCY			ALRARLAS	GW GW GW GW GW GW GW GW GW GW				PI PI PI NI PI PI	S F G S F S S S S S S S S S S S S S S S				PQL VVEYIV VVEYIV	RT PT FS RT RS RT PT	- P PA - C - C - C - C - C - C - C - C - C - C				49 - V - V - V - V - V - V - V - V - V	FVI MI MI FVI FVI LV	- 111111111111111111111111111111111111	140 162 154 149 151 156 159 136 164 159 64 84
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycobacterium tuberculosis [AAC(2')-lc] Mycolicibacterium tarabiense Deinococcus wulumuqiensis Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans	109 108 104 97 99 100 102 105 82 110 105 141 163 155	RV/ QV DE RL TL ETI KI QVI RQV RQV RQV RQV RQV	ARA IRG IG- IRG ID- TH IAS /RG /AR /AR	AGA - AF - DY - GF - EF - EF - CY - AY - GY		GA GA GA GA GA GA GA GA GA GA GA GA GA G		GD AESSAED SAESD SAESD ALSSA ALSSA		PE D D R R R R R R R R R R 	AEA SRF - VV GAA - HE GQF GQF GQL GQL ARF GQL C C C C C C C C C C C C C C C C C C C			ALRARLV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV ARRALV A	GW GW GW GW GW GW GW GW GW GW GW GW GW				PI PI PI NI FI PI	F F G S F S S S S S S S S S S S S S S S				PQL VVEY VVEY VVEY VVEY VVEY VVEY VVEY	RT PT FS LT RS RT PT	- P P A - C P - C - C - C - C - C - C - C - C - C - C				49 - V - V - V - V - V - V - V - V - V	FVI MI MI FVI FVI LV	- 111111111111111111111111111111111111	140 162 154 149 159 156 159 136 164 159 136 164 159
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-Ia] Mycolabacterium tuberculosis [AAC(2')-Ic] Mycolicibacterium arabiense Deinococcus wilumuqiensis Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae	109 108 104 97 99 100 102 105 82 110 105 141 165 150	RV/ QV DE RL ETI KI QVN RQV RQV RQV RQV	ARA IRG IG- IRG ID- TH IAS /RG /AR /AR /AR	AGA - AF - DY - GF - EF - EF - CY - AY - GY		AA GA GA GA GA GA GA GA GA GA GA CPP'		GD AESSAED SAESD SAESD SAESD ALSSD ALSSD		PEI - D - D - R - A - E SAC	AEA SRF - VV GAA - HE GQA ARF GL(GLA RC) DWF			ASRARLSSRAAG						F G S F S S S S S S S S S S S S S S S S					RT PT FS LT RS RT PT	P P A - C P - C - C - C - C - C - C - C - C - C - C				49 - V - V - V - V - V - V - V - V - V - V	FVI MI MI FVI FVI LV	- MLLYYWLLYY	140 162 154 149 151 156 159 136 164 159 64 84 84 73 73
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders Likely binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycolicibacterium arabiense Deinococcus wulumuqiensis Deinococcus wulumuqiensis Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii	109 108 104 97 99 100 102 105 82 110 105 82 110 105 141 163 155 150 152	RV/ QV DE RL ELTI KQVN RQV RQV RQV RQV RQV RQV RQV RQV RQV RQV	ARA IRG IG- IRG ID- TH IAS /RG /AR /AR /AR	AGA - AF - DY - GF - EF - EF - CY - AY - GY		AA GA GA GA GA GA GA GA CA CA CA CA CA CA CA CA CA CA CA CA CA		GDESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAESS			AEA SRF - VI GAA - HE GQP GQL GQL RCI DWF DWF			ALRARLASSRALG					PI PI PI NI PI PI	F G S F S S T S T S T S T S T S T S T S T S					RT PT FS RT RT PT	P - P - C - C - P - T - P - T - P - T - P				49 - V - V - V - V - V - V - V - V - V - V	F VI MII MIV F VI F VI L V	- M L L Y Y W L L Y Y	140 162 154 149 149 159 156 159 136 164 159 136 164 159 136 164 159 136 164 159 136 164 159 136 164 159 136 162 159 136 162 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 136 159 159 136 159 159 159 159 159 159 159 159 159 159
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders Likely binders Bindere	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycobacterium tuberculosis [AAC(2')-la] Mycolicibacterium tarebiense Deinococcus wulumuqiensis Deinococcus wulumuqiensis Deinococcus wulumuqiensis Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la]	109 108 104 97 99 100 102 105 82 110 105 82 110 105 1155 1150 1150 1152 1157	RV/ QV DE RL TL ETI KI QV RQV RQV RQV RQV RQV RQV RQV RQV RQV	ARA IRG IG- IRG ID- TH IAS MRG MRG /AR /AR	AGA - AF - DY - GF - EF - EF - CY - AY - GY - GY		AA GA GA GA GA GA GA GA GA CA CA CA CA CA CA CA CA CA CA CA CA CA		GDESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAEDAESSAESS		PE-I -D -R -R -E -C -C -C -C -C -C -C -C -C -C -C -C -C	AEA SRF - VI GAA - HE GQL GQL RCI DWF DWF DWF DF			ARLLVRREG SAAG						FI S FI S S T S T S T S T S T S T S T S T S T					RT PT FS LT RS RT PT	P P A C - C - C - C - C - C - C - C - C -				49 - V - V - V - V - V - V - V - V	FVI MIL MGVI FVI LV	- MLLYYWLLYY	140 162 154 149 151 156 159 136 164 159 164 159 164 159 164 159 164 159 164 159 164 159 164 159 173 180 73 80 78
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders Likely binders Binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Providencia stuartii [AAC(2')-Ia] Mycobacterium tuberculosis [AAC(2')-Ic] Mycolicibacterium arabiense Deinococcus wilumuqiensis Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-Ia] Mycobacterium tuberculosis [AAC(2')-Ic]	109 108 104 97 99 100 102 105 82 110 105 141 163 150 152 157 160	RV/ DE RL TL ETI KU QVV RQV RQV RQV RQV RQV RQV RQV RQV RQV	ARA IRG IG- IRG ID- TAS ARG /RG /AR /AR /AR	AGA - AF - DY - GF - EF - EF - EF - AY - AY - AY - AY - AY - AY - AY - AY		AA GA GA GA GA GA GA GA GA GA GA GA GA G	LQT LSS LSP LAA LSP LQP LSS LSS LSA LSS LSS LSA LSS LSS LSS LSS	GAESAEDDAESS ATSALGKETAA			AEA SRF GAA - HE GQA ARF GGL GGL RC DWF DWF DWF DWF DWF DWF			ARRESPARES						F F G S F S S S S S S S S S S S S S S S					RTP FS LT RS RT PT	P P A C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - C P - P -				49 - V - V - V - V - V - V - V - V	FVI MIL MGVI FVI LV	- MLLYYWLLYY	140 162 154 149 159 156 159 156 159 164 159 164 184 73 80 78 80 78 81
Non-binders Unlikely binders Likely binders Binders Plasmid-encoded Non-binders Unlikely binders Likely binders Binders	Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycolabacterium tuberculosis [AAC(2')-lc] Mycolicibacterium arabiense Deinococcus sp. NW-56 Cellulosimicrobium cellulans Mycolicibacter senuensis Chioroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii [AAC(2')-la] Mycolocibacter loaumannii Providencia stuartii [AAC(2')-la] Mycolocibacterium tuberculosis [AAC(2')-le] Mycobacterium tuberculosis [AAC(2')-le] Mycobacterium tuberculosis [AAC(2')-le] Mycobacterium tuberculosis [AAC(2')-le] Mycobacterium tuberculosis [AAC(2')-le]	109 108 104 97 99 100 102 105 82 110 105 82 110 105 141 163 155 150 152 157 160 137	RV/ QV DE RL TL ETI KQVV RQV RQV RQV RQV RQV RQV RQV RQV RQV	ARA IRG IG- IRG ID- TAS ARG /RG /AR /AR /AR	AGA - AF - DY - GF - EF LEY - AY - AY - GY - AY - GY - AY - GY		AA GA GA GA GA GA GA GA GA GA GA GA GA G		GASSAEDDAESS ATNALGAAG		PE	AEA SRF GAA - HE GQA ARF GGL ARF GGL ARF GGL ARF GGL A RC DWF DWF DWF DWF DWF DWF DWF			ASRARLVSSAAG						F F G S F S S S S S S S S S S S S S S S					RT PT FS LT RT PT	P P A C P - C - C - C - C - C - C - C - C - C - C				49 - V - V - V - V - V - V - V - V - V - V	FVI MI MU FVI FVI LV	- MLLYYWLLYY	140 162 154 149 159 156 159 136 159 136 164 159 164 184 184 173 73 80 78 81 62
Non-binders Unlikely binders Binders Plasmid-encoded Non-binders Unlikely binders Likely binders Binders Plasmid-encoded	Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii (AAC(2')-la) Mycobacterium tuberculosis (AAC(2')-lc) Mycolicibacterium arabiense Deinococcus wulumuqiensis Deinococcus wulumuqiensis Deinococcus wulumuqiensis Cellulosimicrobium cellulans Mycolicibacter senuensis Chloroflexus aggregans Klenkia soli Vibrio cholerae Acinetobacter baumannii Providencia stuartii (AAC(2')-la) Mycolicibacterium tuberculosis (AAC(2')-lc) Mycolicibacterium arabiense Deinococcus sp. NW-56	109 108 104 97 99 100 102 105 82 110 105 82 110 105 141 165 150 152 157 160	RV/ QV DE RL TLT KQV RQ RL R F F F F F F F F F F F F F F F F F	ARA IRG IG- IRG ID- IAS MRGG /AR /AR /AR /AR	AGA - A F - D Y - G F - E F - E F - E F - A Y - A F - E F - A Y -		GA GA GA GA GA GA GA GA GA GA GA GA GA G	LQT LSS LSP LASP LSS LSS LSS LSS LSS LSS LDT LDT LDT LDT LDT LDT LDT LDT LDT LDT	GAESSAEDDASSEDD LLSAGQUL		PEI-D 	A E / S R F / S R F / S R F / S R F / S R F / S R F / S R F / S R F / S R F / S R F / S R F / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C / S R C /			ASRARLORREG S						F F G S F I S I S I S I S I S I S I S I S I S					RT P-FS RT RT PT	P P A C - C - C - C - C - C - C - C - C -				49 - V - V - V - V - V - V - V - V - V - V	FVI MI MU FVI LV	- MLLYYWLLYY	140 1624 159 156 159 164 159 164 173 80 78 81 62 86 81

Figure S2: Alignment of a subset of sequences based on binding classification. The MAFFT sequence alignment highlights overall sequence identity in blue. Residues important for aminoglycoside binding are denoted with an arrow. Identified residues are numbered as per AAC(2')-Ia from *Providencia stuartii*.

	AAC(2')-Iahis • CoA
Resolution range (Å)	52.75-1.3
	(1.346-1.3)
Space group	P 43 21 1
Unit cell (Å, °)	58.7 58.7 120.0
Total reflections	632665 (60458)
Unique reflections	52409(5117)
Multiplicity	12.1 (11.8)
Completeness (%)	100.0 (99.9)
Mean I/sigma(I)	28.1 (2.6)
Wilson B-factor	19.9
R-merge	0.045 (1.58)
R-meas	0.047 (1.65)
CC _{1/2}	1.00 (0.65)
CC*	1.00 (0.89)
Refinement Statistics	
Rwork	0.148 (0.206)
Rfree ^a	0.189 (0.234)
Number of non-hydrogen atoms	1982
macromolecules	1726
ligands	65
water	191
Protein residues	182
RMS (bonds)	0.030
RMS (angles)	2.4
Ramachandran favored (%)	94.4
Ramachandran outliers (%)	1.1
Clashscore	12.63
Average B-factor	29.9
macromolecules	28.0
ligands	38.0
solvent	44.3

Table S1: Data Collection and Refinement Statistics of AAC(2')-Ia C-terminal His Complex. Statistics for the highest-resolution shell are shown in parentheses. ^{*a*} R_{free} was calculated by randomly omitting 10% of observed reflections from refinement.