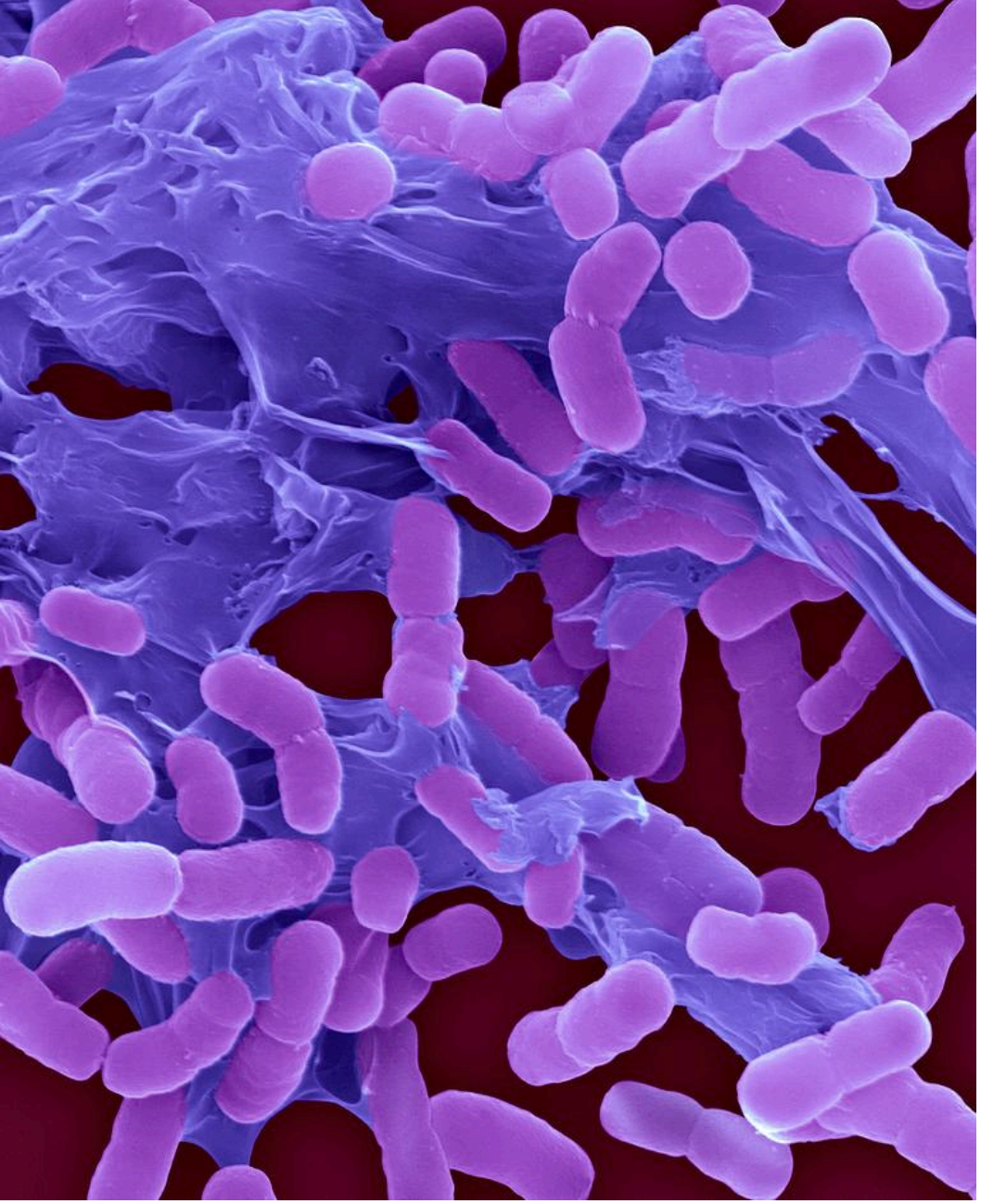

Acinetobacter baumannii is a photograph by Dennis Kunkel Microscopy/science Photo Library which was uploaded on September 23rd, 2018.

Mutational analysis of AdeB transporter function

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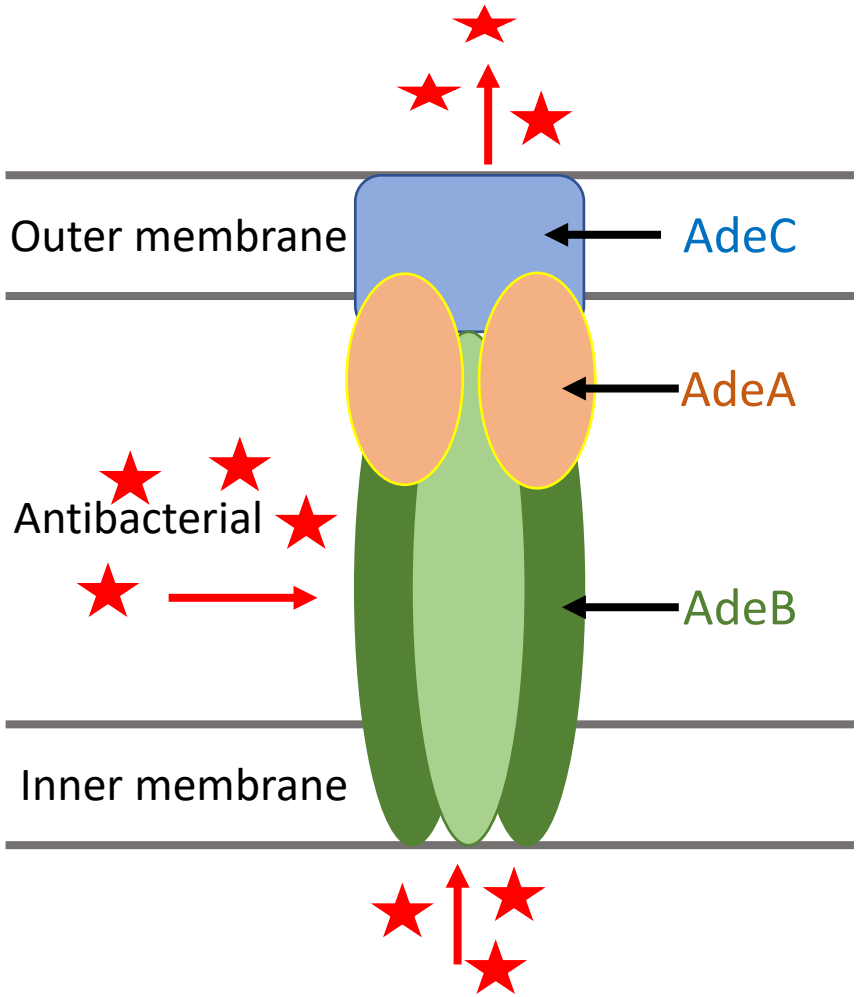
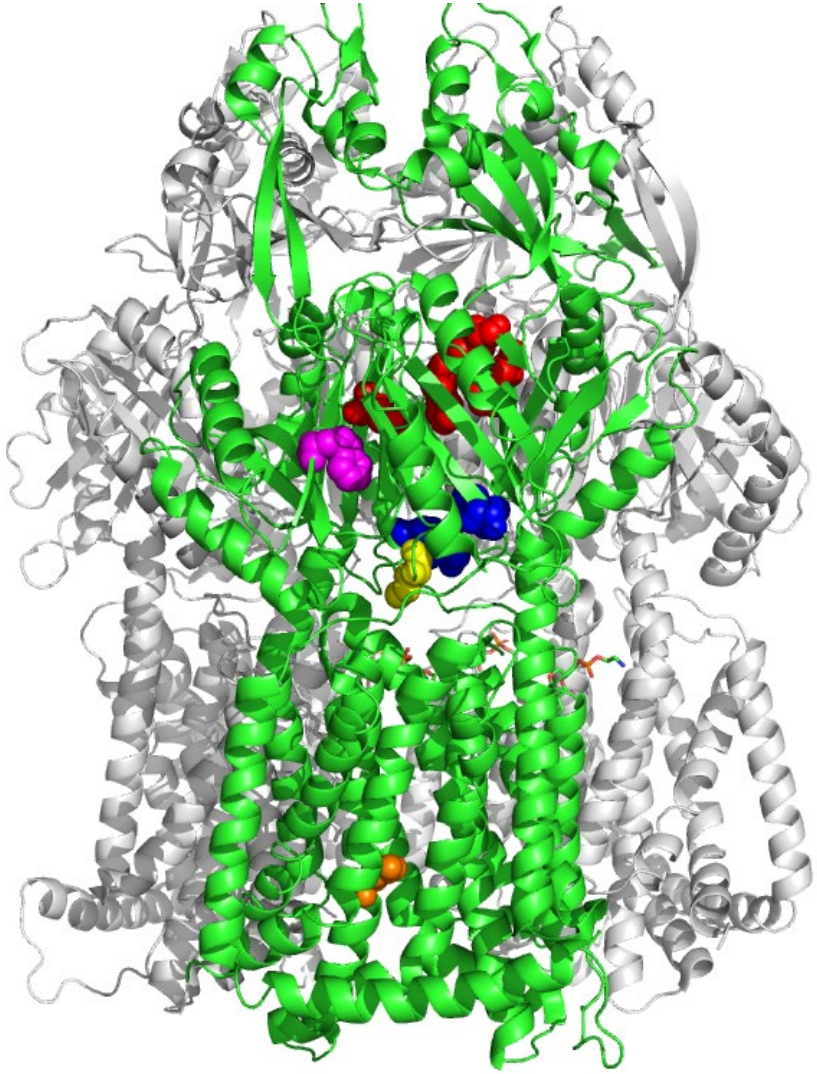


Figure 1. A schematics of AdeABC efflux pump in the cell envelope of *A. baumannii.* The *adeA*, *adeB* and *adeC* genes form an operon, encoding a membrane fusion protein, a multidrug transporter and an outer membrane channel, respectively.

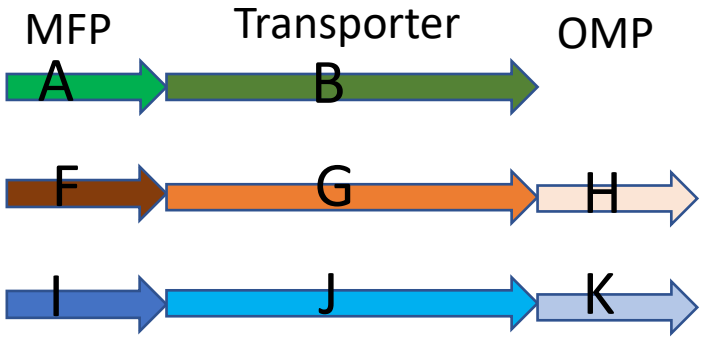


We targeted:
(1) E89, F178, F277, and W610 from the two distal binding sites;
(2) I663 from the conserved flexible loop connecting the cleft entrance to the proximal drug-binding pocket;
(3) W568, D664 and E665 from the proximal multi-drug binding site;
(4) W708 located at the entrance of the periplasmic cleft;
(5) N932 involved in the proton relay network

Figure 2. Structure of AdeB trimer with mutated residues indicated

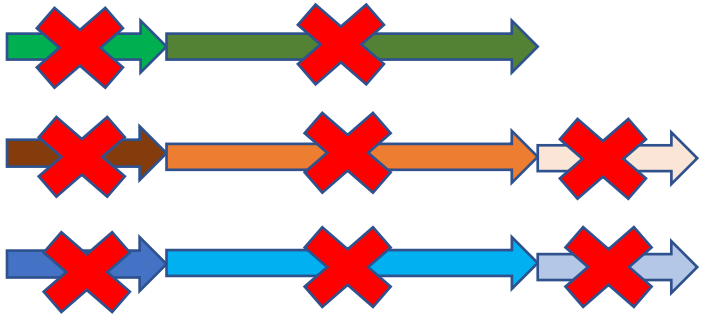
Christopher E Morgan, Przemyslaw Glaza, **Inga V Leus**, Anhthu Trinh, Chih-Chia Su, Meng Cui, Helen I Zgurskaya, Edward W Yu. Cryoelectron Microscopy Structures of AdeB Illuminate Mechanisms of Simultaneous Binding and Exporting of Substrates Mbio, 2021
DOI: 10.1128/mBio.03690-20

RND efflux pumps

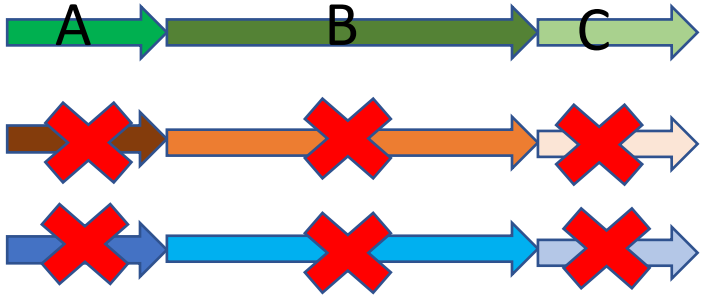


Ab17978
WT strain

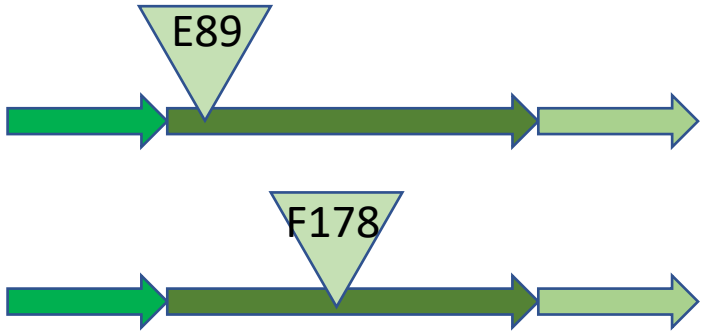
Multiple efflux deficient strain
(AbΔ3 strain)



AdeABC from (Ab AYE)
Overexpression in AbΔ3 strain



Site-directed mutagenesis in
AdeB



Experimental design

Leus IV, Weeks JW, Bonifay V, Smith L, Richardson S, Zgurskaya HI. Substrate specificities and efflux efficiencies of RND efflux pumps of Acinetobacter baumannii. J Bacteriol. 2018;200(13): e00049-18. doi:10.1128/JB.00049-18

Table 1. Minimal inhibitory concentrations (MICs) for *A. baumannii* strains with AdeB carrying the indicated aminoacid substitutions.

AdeB variants	EtBr	Gentamicin	Zeocin	Azithromycin
Δ3	4-8	8-16	1	0.64
AdeB	32-64	32	16-32	10-20
F178C	16-32	64-128	>256	10
W610C	32-64	8-16	16	10 -20
I663C	32-64	8-16	8-16	10
D664C	16-32	16	4-8	2.5-5
E665A	16-32	16	8	10
W708C	32	8-16	8-16	10
N932C	32	16	4	5-10
E89A	32-64	8	32	20
F277C	16	16-32	32	10-20
W568C	32	16	32	20

D644C mutant is hypersusceptible to macrolides

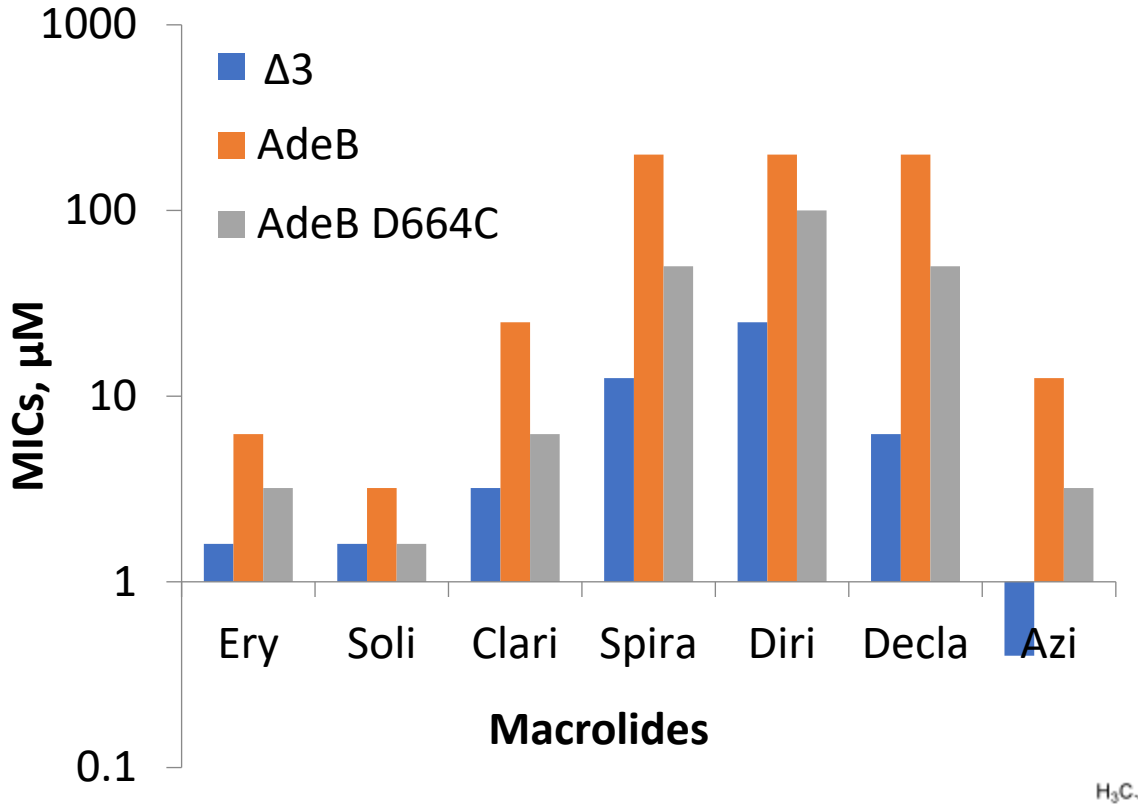
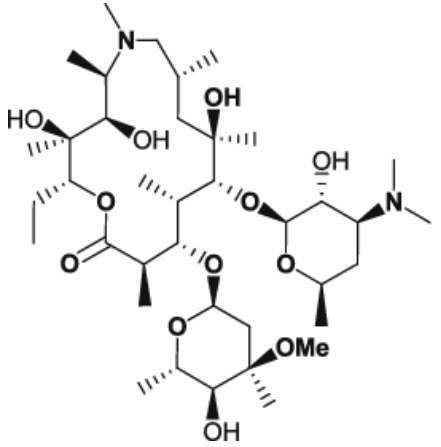
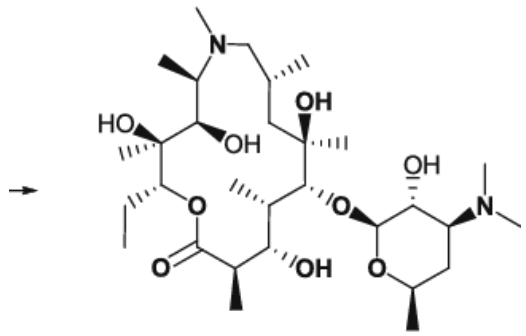


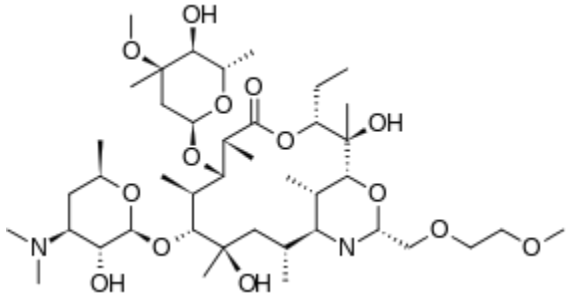
Figure 3. MICs of representative macrolides. Antibiotics are abbreviated as the following: Ery, erythromycin; Soli, solithromycin; Clari, clarithromycin; Spira, spiramycin; Diri, dirithromycin; Decla, Descladinose azithromycin; Azi, azithromycin



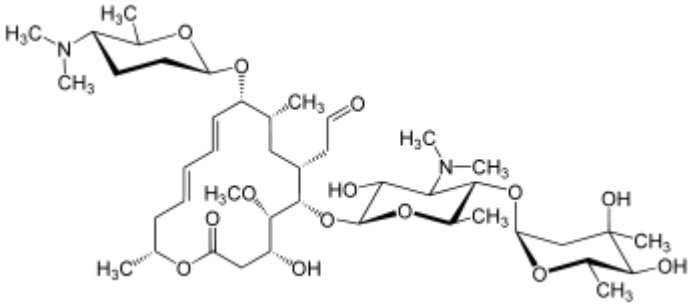
azithromycin



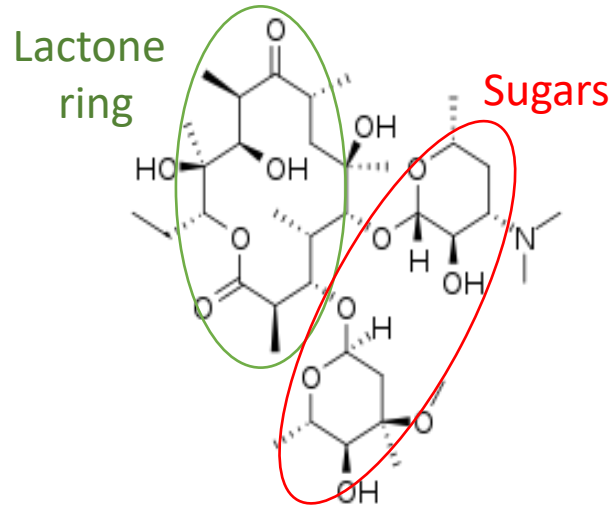
Descladinose azithromycin



dirithromycin

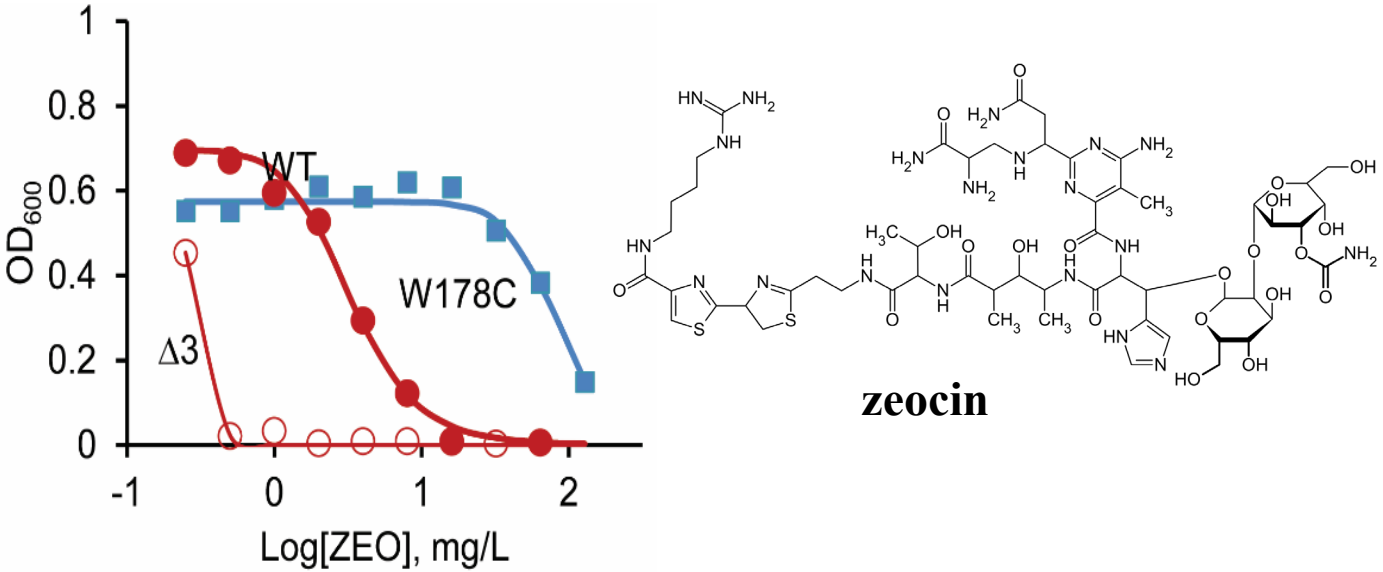


spiramycin



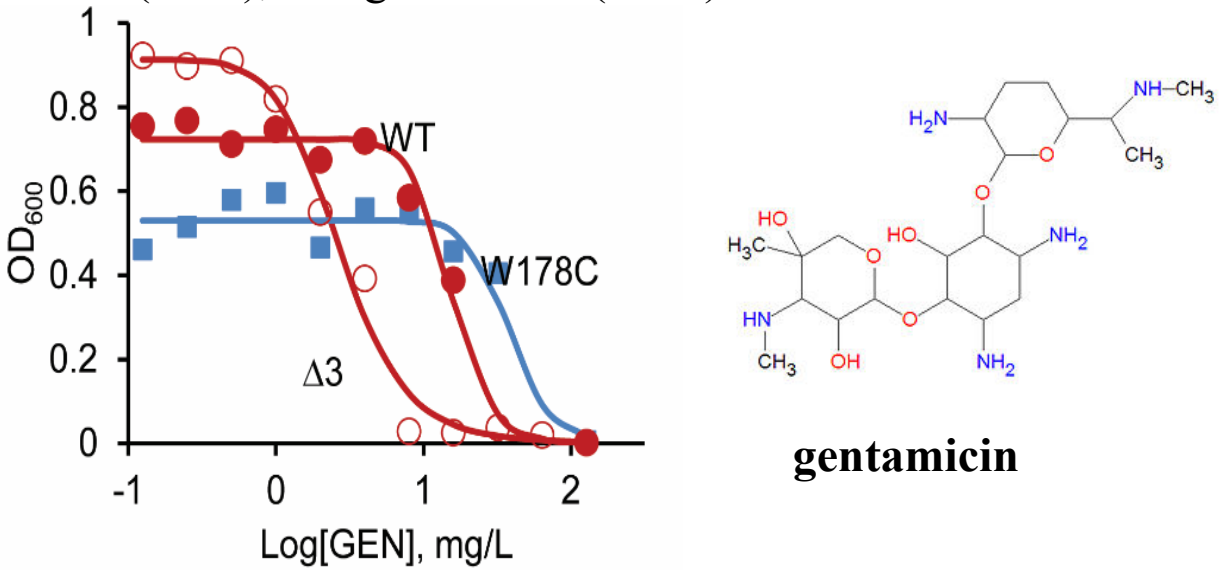
erythromycin

F178C substitution enhanced efflux of gentamicin and zeocin



zeocin

Figure 4. Concentration-dependent inhibition of growth by zeocin (ZEO), and gentamicin (GEN).



gentamicin

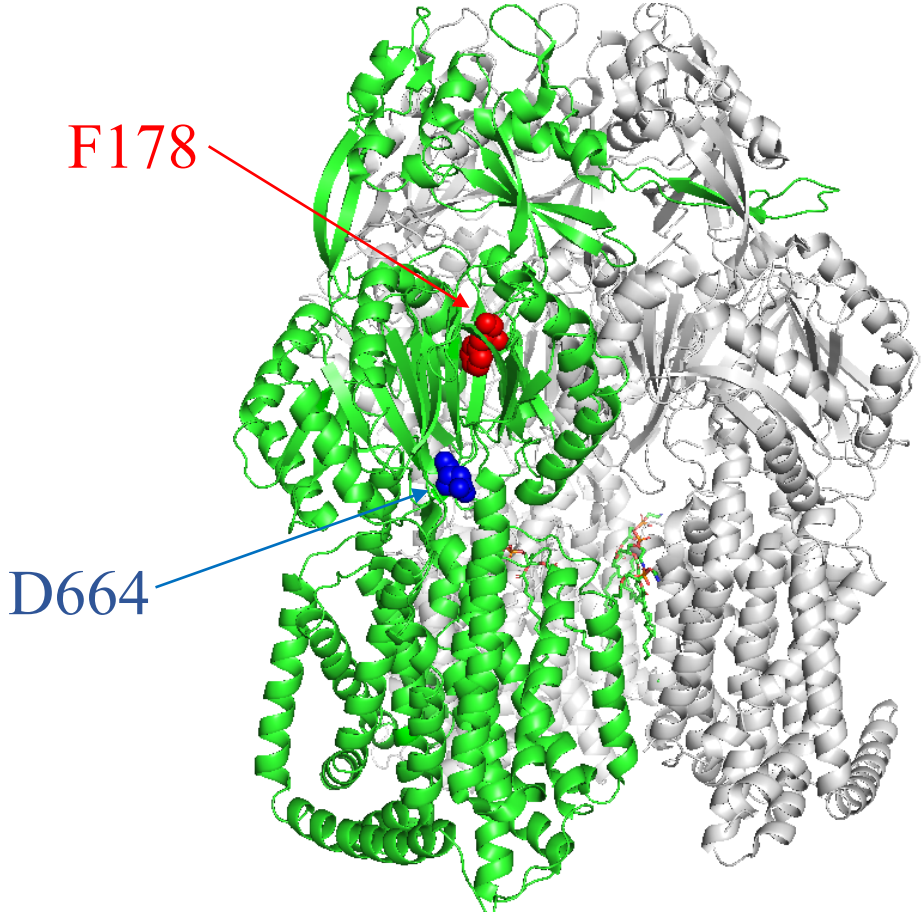


Figure 5. AdeB trimer structure with the amino acid substitutions highlighted in one of the AdeB protomers (green).

Conclusions:

- Out of ten mutated AdeB variants containing single amino acid substitutions, only F178 and D664 residues were identified to be crucial for the function of the pump
- D644C mutant with a substitution in the proximal multi-drug binding site was more susceptible to structurally diverse macrolides
- F178C substitution in the distal binding site enhanced protection against gentamicin and zeocin
- Our results provide a novel insight into the mechanism of AdeB and demonstrate that this transporter is an attractive target for pharmacological development.

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