Supplementary material

A. Shaheen, W. A. Afridi, S. Mahboob, M. Sana, N. Zeeshan, F., O. Mirza3, M. Iqbal, M. Rahman "Reserpine is the new addition into the repertoire of AcrB efflux pump inhibitors" (*Mol. Biology, 2019*)

Table 1S: Various concentrations of ciprofloxacin and reserpine used in disc diffusion assay against pMR4/C41(DE3) and pMR4-AcrB/C41(DE3) *E. coli* cells.	
Disc 1	6 µL of 0.625 µg/mL ciprofloxacin (=3.75 ng) + 4 µL of 30µg/mL reserpine (=120 ng)
Disc 2	6 µL of 0.625 µg/mL ciprofloxacin (=3.75 ng) + 4µL DMSO
Disc 3	6 µL of 1.25 µg/mL ciprofloxacin (=7.5 ng) + 4µL DMSO
Disc 4	6 µL of 1.25 µg/mL ciprofloxacin (=7.5 ng) + 4µL of 30µg/mL reserpine (=120 ng)
Disc 5	6 µL of Water + 4µL of 30µg/mL reserpine (=120 ng)
Disc 6	6 µL of Water + 4 µL DMSO

Table 2S. Various concentrations of ciprofloxacin and reserpine used in time kill assay.			
Falcon-1	Falcon-2	Falcon-3	Falcon-4
9.5mL inoculum (OD_{600}=0.1) + 250µL reserpine (30µg/mL) (=7.5µg in 10mL) + 250µL ciprofloxacin (1.25 µg/mL) (=0.3125µg in 10mL)	9.5mL inoculum (OD_{600}=0.1) + 250µL reserpine (30µg/mL) (=7.5µg in 10mL) + 250µL water	9.5mL inoculum (OD_{600}=0.1) + 250µL DMSO + 250µL ciprofloxacin (1.25µg/mL) (=0.3125µg in 10mL)	9.5mL inoculum (OD_{600}=0.1) + 250µL DMSO + 250µL water

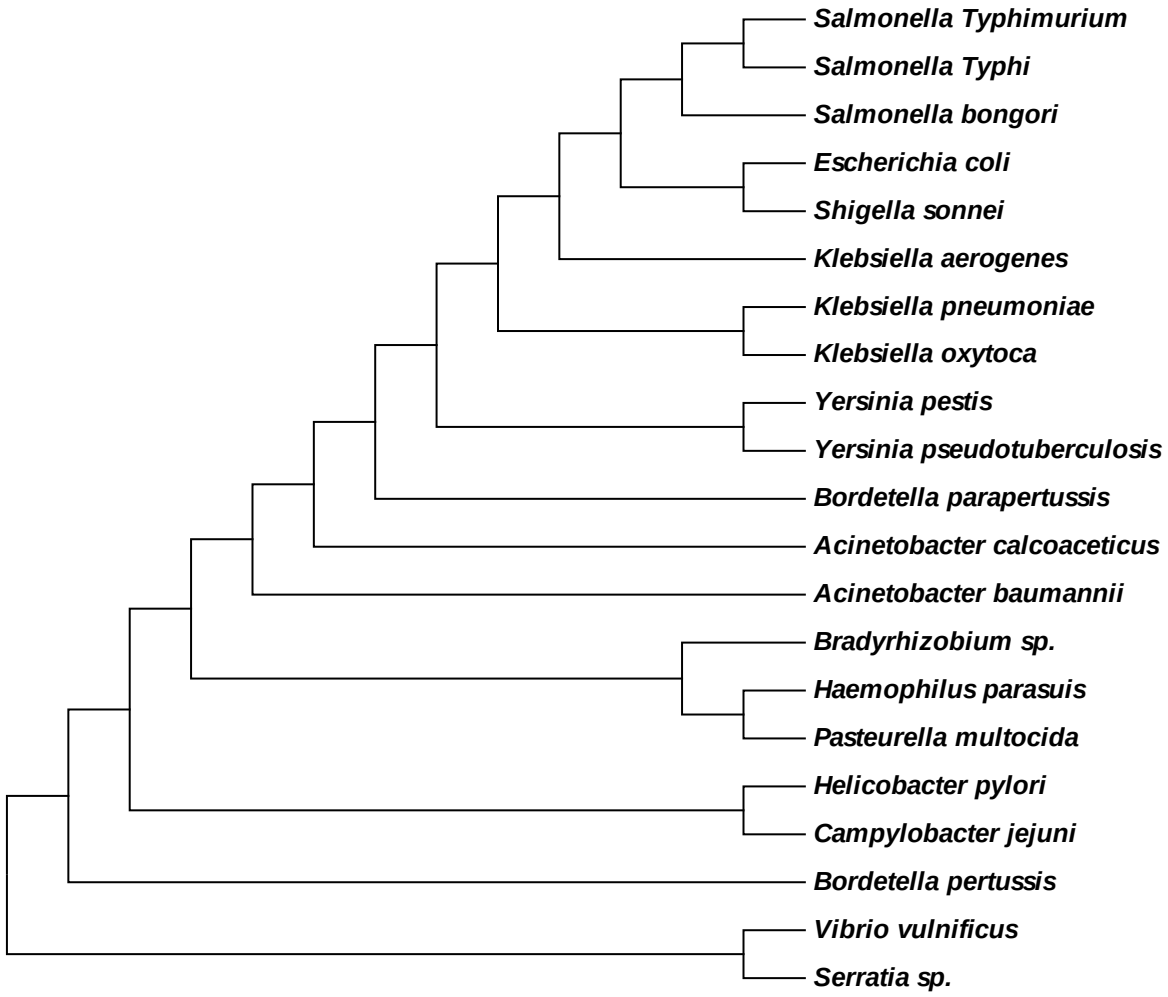
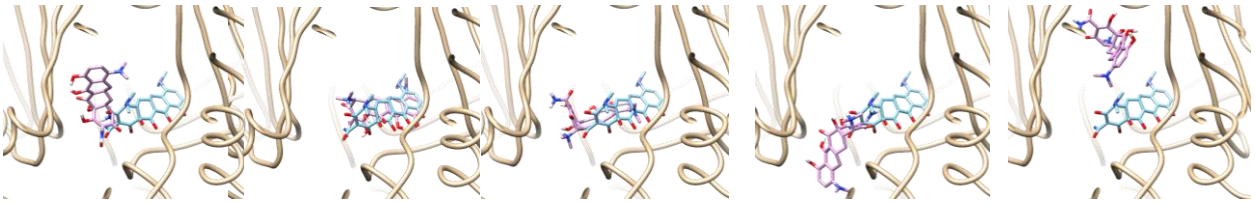


Figure1S. Evolutionary relationships of *S.* Typhi AcrB with other AcrB sequences in Gram negative bacteria. All sequences were retrieved from Uniprot and Genbank databases.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 5.90270263 is shown. The evolutionary distances were computed using the Poisson correction method [2] and are in the units of the number of amino acid substitutions per site. The analysis involved 21 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 580 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [3].



Pose 1 Pose 2 Pose 3 Pose 4 Pose 5

Figure 2S: Top five poses for docked minocycline shown in purple. Crystallized minocycline is with blue carbon skeleton.

Table 3S. Predicted binding energies (in kcal) of all 9 poses for minocycline, ciprofloxacin and reserpine with S. Typhi AcrB

Ligands	**Pose 1**	**Pose 2**	**Pose 3**	**Pose 4**	**Pose 5**	**Pose 6**	**Pose 7**	**Pose 8**	**Pose 9**
Minocycline	-8.7	-8.1	-7.8	-7.7	-7.5	-7.3	-7.3	-7.2	-7.1
Ciprofloxacin	-8.5	-7.7	-7.6	-7.6	-7.5	-7.4	-7.3	-7.2	-7.1
Reserpine	-9.4	-9.0	-9.0	-8.7	-8.7	-8.6	-8.5	-8.5	-8.4

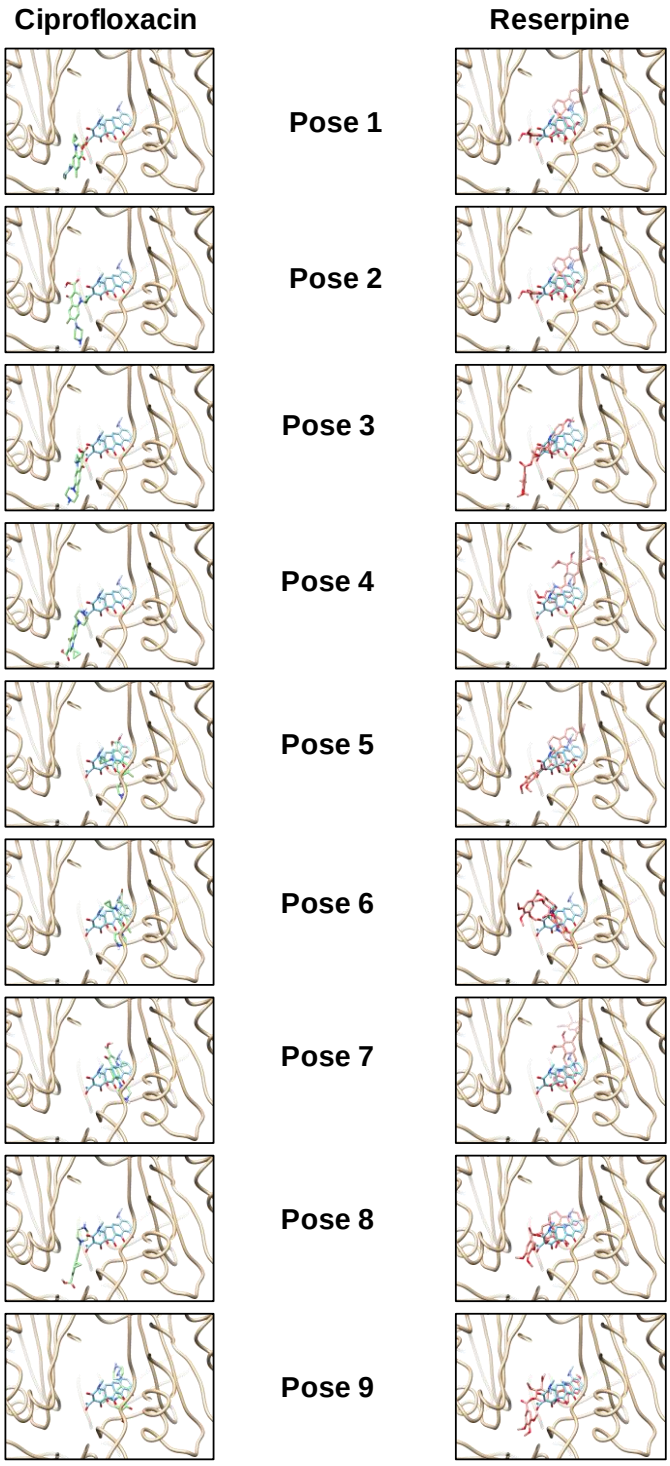


Figure 3S: Docking results for ciprofloxacin and reserpine; when compared with crystallized minocycline shown in blue, both ciprofloxacin and reserpine occupied groove binding area and none of the pose was found to be in large pocket.

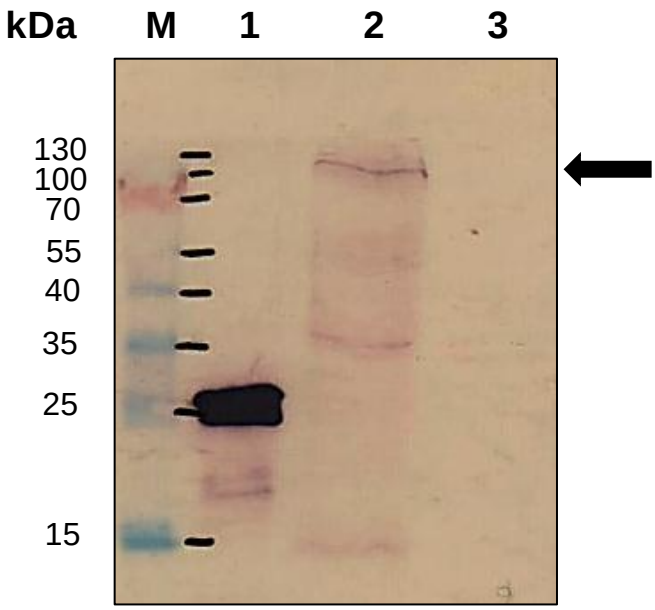


Figure 4S Western blot analysis of expression of the AcrB protein in *E. coli* cell transformed with pMR4-AcrB. Membrane samples of *E. coli* cells transformed with respective plasmids (20 µg) (expression induced by auto-induction and cell membranes prepared by water lysis method) were subjected to SDS-PAGE followed by Western blotting for His-tag. His-tag detection was made using primary antibody against oligohistidine tag and secondary mouse IgG antibody conjugated to alkaline phosphatase. Lane 1 shows oligohistidne-tagged kinase of *Mycobacterium tuberculosis* (positive, ~25kDa), lane 2 represents the oligohisidine-tagged AcrB protein band of ~100 kDa (arrowed) and lane 3 represents cell lysate of control cells transformed with pMR4. 'M' represents the prestained Protein Marker (Thermo Scientific Catalog # 26616).

Note: Protein maker bands of 130, 100 and 55 kDa are not visible on the blot as these are faded during the process of western blot development. Also marker band of 180 kDa was not transferred on the membrane (blot) presumably due to high molecular weight and is not shown. Black lines on blot were drawn just after protein transfer before blot development.

References

1. Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

2. Zuckerkandl E. and Pauling L. (1965). Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson and H.J. Vogel, pp. 97-166. Academic Press, New York.

3. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution 33:1870-1874.