Resistance frequency in *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* exposed to BWC0977, a novel bacterial topoisomerase inhibitor

Sreevalli Sharma, Savitha Raveendran, Dhanashree Pillai, Akshaya Ravishankar, Vasan Sambandamurthy, Nainesh Katagihallimath, Santanu Datta, V Balasubramanian, Vasanthi Ramachandran

Bugworks Research India Pvt. Ltd. - Bangalore (India)

P2133

sreevalli@bugworksresearch.com

Background

Resistance to novel bacterial topoisomerase inhibitors (NBTI) reported so far arises from spontaneous mutations in chromosomal genes of the target enzymes: DNA gyrase and topoisomerase IV. We describe here the resistance frequency and characterisation of spontaneous resistant mutants in Gram negative bacteria against BWC0977.

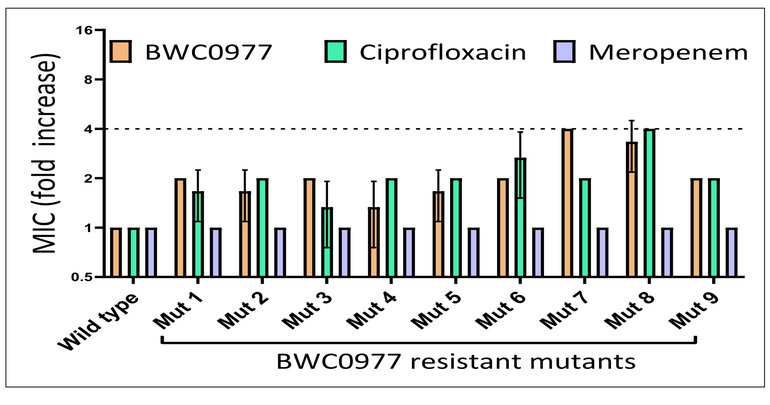
Methods

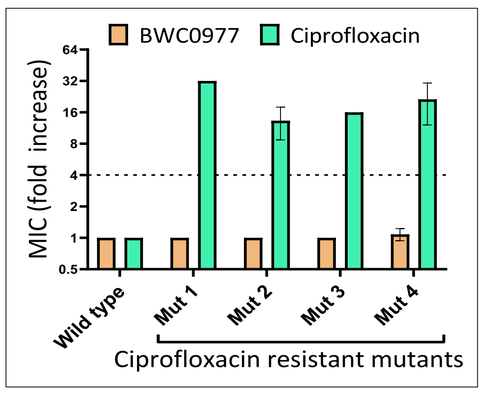
Mid-logarithmic phase cultures of Gram-negative bacteria (10^9 CFU/mL) were plated on Luria-Bertani agar plates containing BWC0977 equivalent to 4x, 8x and 16x MIC. Plates were incubated for 24-36 hours at 37^0C and the spontaneous frequency of resistance was calculated. No colonies were obtained in 8x & 16x MIC plates.

Resistance frequency of BWC0977 in Gram negative pathogens

Resistance frequency	*E.co* ATCC 25922		*P.ae* ATCC 27853		*A.ba* ATCC 19606	
	BWC0977	Cipro	BWC0977	Cipro	BWC0977	Cipro
MIC µg/mL	0.06	0.012	0.25	0.5	0.125	0.5
4x MIC	**<1*10^{-9}**	5*10^{-9}	**<1*10^{-9}**	<1*10^{-9}	**2.5*10^{-9}**	8*10^{-8}
8x MIC	**<1*10^{-9}**	<1*10^{-9}	**<1*10^{-9}**	<1*10^{-9}	**<1*10^{-9}**	<1*10^{-9}

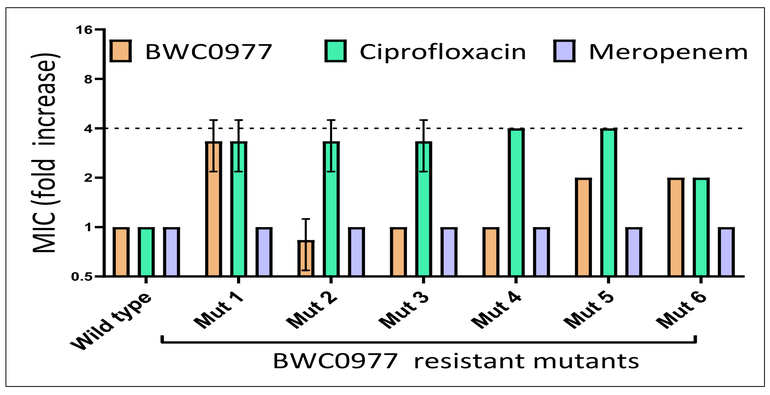
MIC modulation and cross resistance in *E.co* ATCC 25922

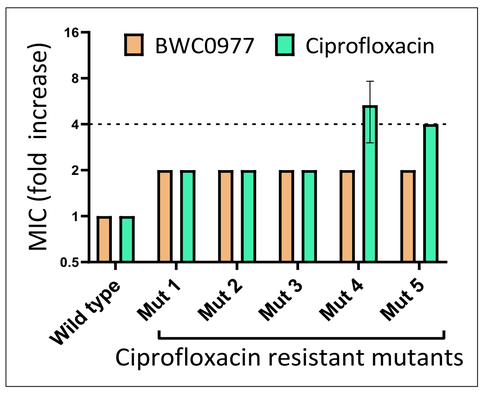




- BWC0977 mutants showed 2- 4 fold increase in MIC. No cross resistance observed with other classes of antibiotics including fluoroquinolones.
- Whereas, resistant colonies raised against ciprofloxacin showed 4-32 fold MIC modulation with fluoroquinolones but were still sensitive to BWC0977.
- Sequencing *gyr*A, *par*C, *gyr*B and *par*E of BWC0977 resistant colonies revealed no mutations in the key NBTI target binding sites.
- BWC0977 Mut-3 and Mut-9 showed mutation corresponding to **T180I** in GyrA. None of had mutations in *par*C, *gyr*B and *par*E.
- 4/4 ciprofloxacin resistant mutants confirmed S83L or D87Y in QRDR region of GyrA.

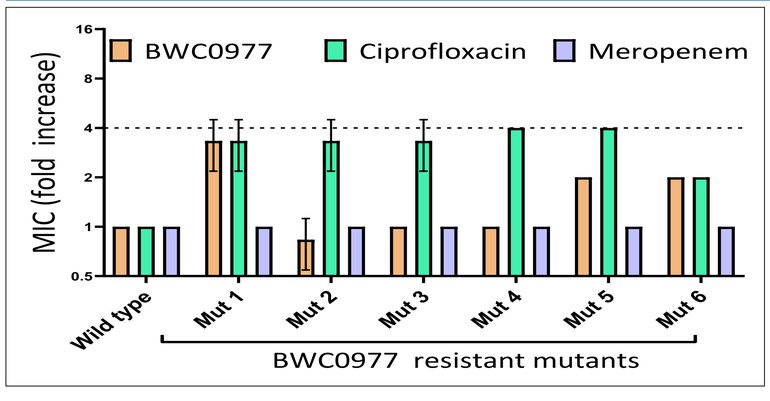
MIC modulation and cross resistance in *P.ae* ATCC 27853

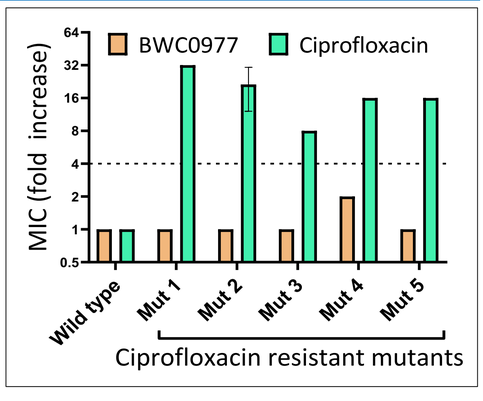




- BWC0977 mutants showed 2- 4 fold increase in MIC.
- No cross resistance observed with other classes antibiotics including fluoroquinolones.
- BWC0977 Mut-1 showed **A179V** and BWC0977 Mut-4 showed **P35S** in GyrA. No mutations were observed in *par*C, *gyr*B and *par*E.

MIC modulation and cross resistance in *A.ba* ATCC 19606





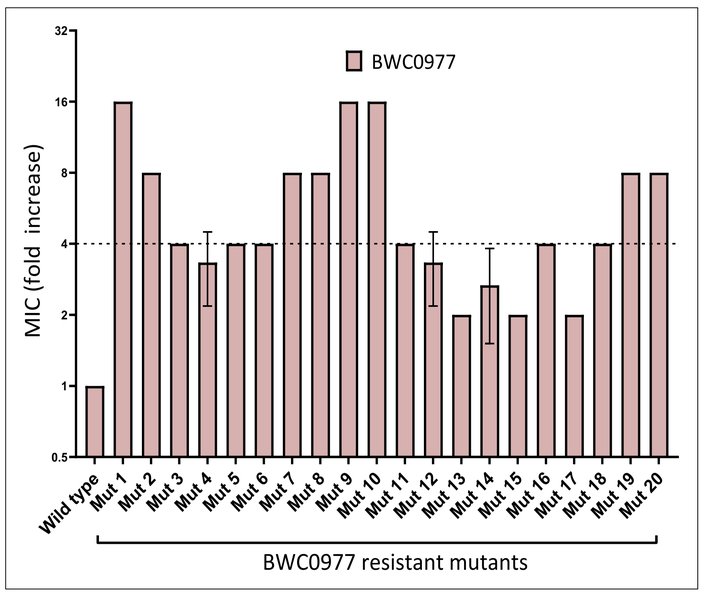
- BWC0977 mutants showed 2- 4 fold increase in MIC.
- None of the mutant strains were cross resistant to other classes antibiotics including fluoroquinolones.
- Whereas, resistant mutants raised against ciprofloxacin showed 4-32 fold MIC modulation with fluoroquinolones but were still sensitive to BWC0977.
- BWC0977 Mut-1 showed **M118R** in GyrA. No mutations were observed in *par*C, *gyr*B and *par*E.
- G79C in GyrA was found in all ciprofloxacin resistant mutants.

Resistance frequency of BWC0977 in multi-drug resistant pathogens

Multi-drug resistant strains from ATCC are clinical isolates with NDM, OXA, KPN and target site mutations conferring resistance to as many as 36 antibiotics of various classes such as carbapenems, β-lactams, cephalosporins, quinolones, tetracyclines, glycylcyclines, aminoglycosides, and dihydrofolate reductase inhibitors. We determined the *in vitro* resistance frequency of BWC0977 and cross resistance profiles in some of these strains.

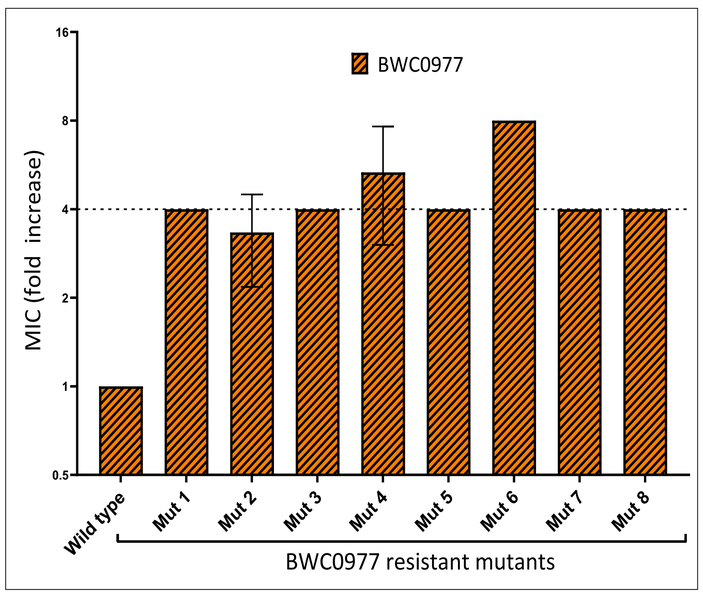
Resistance frequency	*E.co* BAA 2471	*P.ae BAA* 2797	*A.ba* BAA 2885
MIC µg/mL	0.5	0.6	0.3
4X MIC	**4*10^{-9}**	**<1*10^{-9}**	**2.5*10^{-8}**
8X MIC	**<1*10^{-9}**	**<1*10^{-9}**	**<1*10^{-9}**

MIC modulation in MDR *E.co* BAA 2471



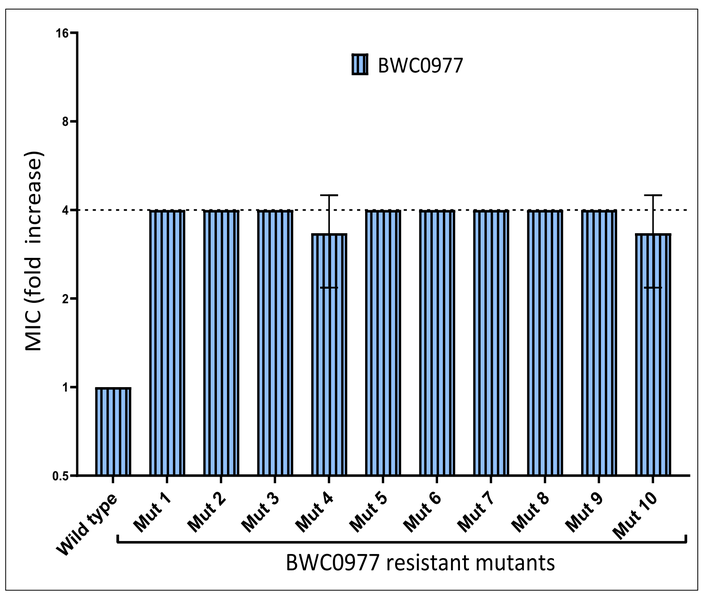
- Mutant colonies showed a 2-16 fold increase in MIC compared to parent strain.
- Parent strain had pre-existing background mutations: S83L & D87N in GyrA, S80I in ParC and S458A in ParE.
- No additional mutations were found in GyrA and GyrB.
- Mut 2, 7, 8 & 20 had additional **D79N** in ParC.
- Mut 10 showed **P439T** and Mut 19, 20 showed **K441I** in ParE.

MIC modulation in MDR *P.ae* 2797



- Mutant colonies showed a 2-8 fold increase in MIC.
- Parent strain had pre-existing background mutations: T83I in GyrA, S87L in ParC and D533E in ParE.
- All 8 mutants had **A179V** in GyrA upon BWC0977 exposure.
- Mut 6 showed additional **D87V** in GyrA.
- No mutations were found in ParC, GyrB and ParE.

MIC modulation in MDR *A.ba* 2885



- Mutant colonies showed a 2-4 fold increase in MIC.
- Parent strain had pre-existing background mutations: S81L in GyrA, S84L, E208G, S467G, A661V in ParC, E479D in GyrB and V237A in ParE.
- No additional mutations were found in GyrA, ParC and ParE.

Conclusions

- *In vitro* resistance frequency of BWC0977 at 4x MIC was found to be <10^{-9} in *Escherichia coli* ATCC 25922 & *Pseudomonas aeruginosa* ATCC 27853 and 2.5*10^{-9} in *Acinetobacter baumannii* ATCC 19606.
- The dual target (GyrA and ParC) inhibition of BWC0977 provides the advantage of very low frequencies of resistance even in multidrug resistant strains.

Acknowledgement and Disclaimer:
Research reported in this presentation is supported by CARB-X. CARB-X's funding for this project is sponsored by the Cooperative Agreement Number IDSEP160030 from ASPR/BARDA and by awards from Wellcome, Germany's Federal Ministry of Education and Research, and the UK Global Antimicrobial Resistance Innovation Fund (GAMRIF) funded by the UK Government Department of Health and Social Care (DHSC). The content is solely the responsibility of the authors and does not necessarily represent the official views of CARB-X or any of its funders