

# In vitro Activity of BWC0977, a Novel Bacterial Topoisomerase Inhibitor, against Molecularly Characterized Enterobacteriaceae & Non-Fermenter Isolates of the CDC collection and key Biodefense Pathogens

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## Introduction

BWC0977 is a dual target NBTI, with potent broad-spectrum activity. Mode of action studies with *E. coli* using radiolabelled precursors demonstrate that BWC0977 inhibits DNA synthesis. Evaluation of mechanism of inhibition show that BWC0977 stabilizes the cleavage complex after the single strand break, thus differing from fluoroquinolones. Further validation of this unique feature is substantiated by the effectiveness of BWC0977 against fluoroquinolone resistant clinical isolates. The current study provides evidence of its potency against multi-drug resistant Gram negative & Gram positive pathogens & bio-defense pathogens.

## Materials & Methods

Broth dilution methods were used to assess the growth inhibitory properties and determine the minimum inhibitory concentrations of the test compound. For members of the Enterobacteriaceae family and the non-fermenters, the standard *in vitro* medium (Ca-MHB) was sufficient as growth and assay medium. For the bio-defense pathogens, specific media (*B. anthracis*-Tryptic soy broth, *F. tularensis*-modified cation adjusted Mueller Hinton broth, *Y. pestis*-Mueller Hinton broth, *B. pseudomallei* and *B. mallei*-Luria Bertani broth) were used for growing the culture, initial inoculum preparation and assay purposes. Standard drug MICs were always run in parallel. The break-point MICs were determined using CLSI guidelines.

## Conclusions

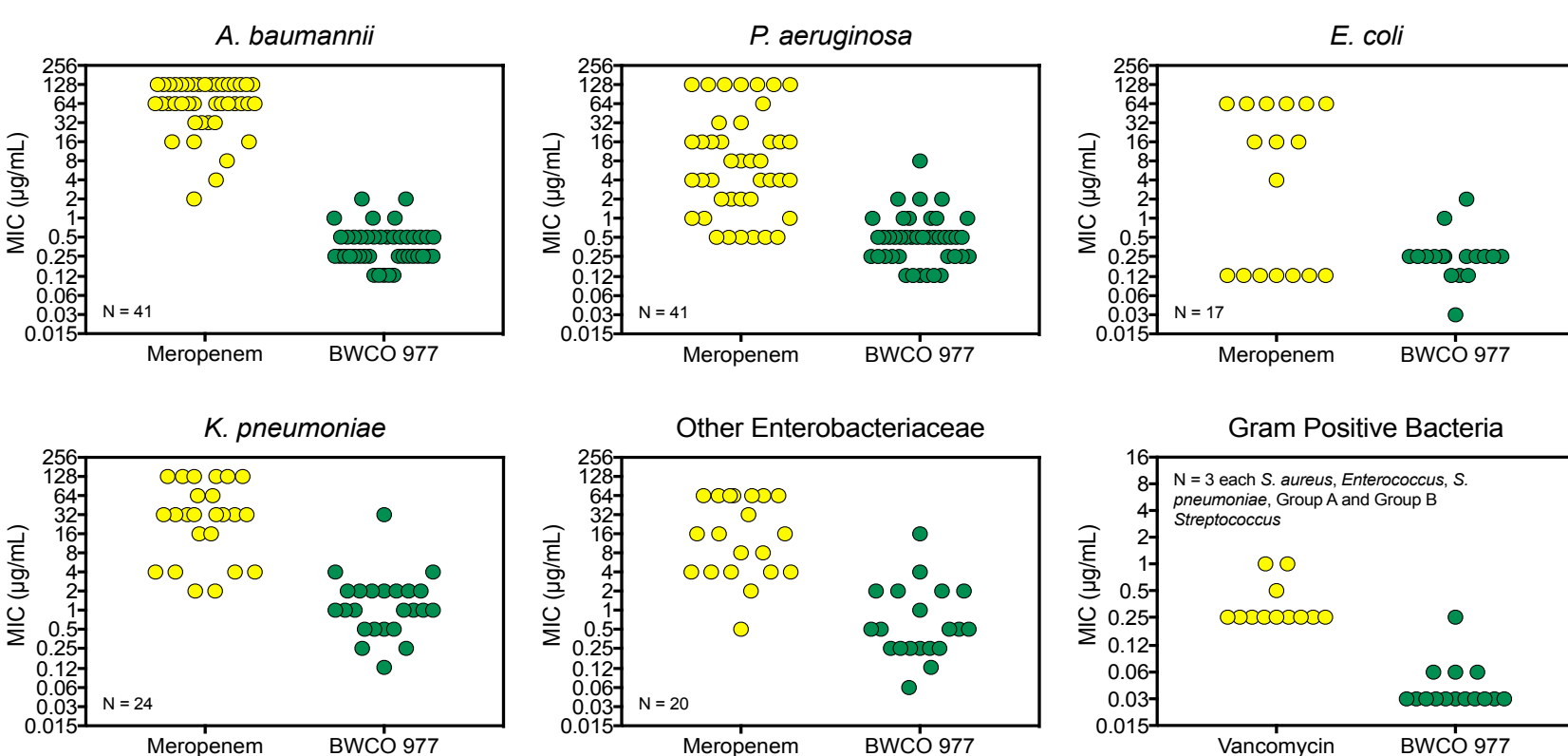
- BWC0977 Is a broad spectrum anti-bacterial agent potent against wide range of drug resistant members from Enterobacteriaceae, non-fermenters and Bio-defense pathogens
- BWC0977 has emerged as a promising pre-clinical candidate and further investigations are underway towards pre-clinical development for treatment of difficult to treat hospital associated infections

## Results

<i>E. coli</i> Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
ATCC 25922 [CLSI control]	≤ 0.125	0.25
AR 348	16	0.25
AR 494, 350 [mcr-1]	≤ 0.125	0.25, < 0.03
AR 346, 349, 493, 495 [mcr-1, ESBL]	≤ 0.125	0.125
AR 114 [KPC-3,TEM-1B]	4	0.25
AR 118 [NDM-1,TEM-1A,CMY-6,OXA-2]	64	0.25
AR 119 [NDM-1,OXA-9,TEM-1B,CMY-6,CTX-M-15, OXA-1, OXA-2]	16	0.25
AR 128 [NDM-1,CMY-6,CTX-M-15,OXA-2]	64	0.25
AR 137 [NDM-6,OXA-9,TEM-1A,CMY-42,CTX-M-15,OXA-1]	64	2
AR 149 [NDM-7,CMY-42]	16	0.125
AR 150 [NDM-5,TEM-1B,CMY-42]	64	0.25
AR 151 [NDM-5,TEM-1B,CMY-42,CTX-M-15,SHV-12,OXA-1]	64	0.25
AR 162 [NDM-7,TEM-1B,CTX-M-15]	64	1

<i>K. pneumoniae</i> , Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
AR 112	32	1
AR 347 [KPC]	>64	2
AR 113 [KPC-3,SHV-11]	>64	1
AR 115 [KPC-3,TEM-1A]	32	2
AR 120 [KPC-2,TEM-1D]	64	1
AR 125 [KPC-3,OXA-9,TEM-1B]	32	1
AR 117 [KPC-3,OXA-9,TEM-1A]	32	4
AR 126 [KPC-2,TEM-1B,OXA-1]	4	0.5
AR 129 [KPC-3,TEM-1A]	>64	2
AR 135 [VIM-1,OXA-9,TEM-1A,SHV-12]	2	2
AR 138 [NDM-7,TEM-1B,CTX-M-15,SHV-11]	>64	4
AR 139 [NDM-1,CMY-4,CTX-M-15,SHV-11,OXA-10]	32	0.5
AR 140, 141, 142 [OXA-181,CTX-M-15,SHV-26]	4	1
AR 143 [NDM-1,OXA-9,TEM-1A,CMY-4,CTX-M-15]	>64	2
AR 145, 152 [NDM-1,OXA-9,TEM-1A,CTX-M-15,SHV-11,OXA-1]	32	1.0 - 2.0
AR 146 [NDM-1,CTX-M-15,SHV-11,OXA-1]	16	0.25
AR 147 [KPC-3,OXY-1-4]	2	0.125
AR 148 [NDM-1,TEM-1B,CMY-6,CTX-M-15,SHV-11,OXA-1]	32	0.5
AR 153 [NDM-1,OXA-232,OXA-9,TEM-1A,CTX-M-15,OXA-1]	>64	2
AR 158 [NDM-1,TEM-1B,CTX-M-15,OXA-1]	64	>16
AR 160 [OXA-48,SHV-11]	16	0.25

Other Enterobacteriaceae, Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
Citrobacter species AR 157, NDM-1,OXA-9,TEM-1B,CTX-M-15	64	2
Citrobacter freundii AR 116, KPC-2,CMY-79,CMY-76	16	0.5
Enterobacter aerogenes AR 161, IMP-4,TEM-1B,OXA-1,SFO-1	4	0.25
Enterobacter cloacae AR 154, VIM-1,TEM-1B,ACT-7	4	2
Enterobacter cloacae AR 163, KPC-2,TEM-1B,ACT-7,CTX-M-15,OXA-1	4	16
Enterobacter cloacae AR 132, NMC-A	16	0.25
Enterobacter cloacae AR 136, KPC-3,OXA-9,TEM-1A,SHV-12	16	0.5



Parameter	<i>A. baumannii</i>		<i>P. aeruginosa</i>		<i>E. coli</i>		<i>K. pneumoniae</i>		Enterobacteriaceae	
	Meropenem	BWC0977	Meropenem	BWC0977	Meropenem	BWC0977	Meropenem	BWC0977	Meropenem	BWC0977
Range	2 - >64	0.125 - 2	0.5 - >64	0.125 - 8	< 0.125 - 64	< 0.03 - 2	2 - >64	0.125 - >16	0.5 - 64	0.06 - 16
MIC50	64	0.25	8	0.5	4	0.25	32	1	8	0.5
MIC90	64	0.5	>64	1	64	0.25	>64	4	64	2
GM MIC	58.8	0.36	7.23	0.46	3.26	0.24	25.4	1.16	13.5	0.61

## Activity against Bio-defense pathogens

Organism	Strain	MIC (µg/ml)		Organism	Strain	MIC (µg/ml)		Organism	Strain	MIC (µg/ml)	
		BWC0977	Doxy/Caz			BWC0977	Doxy/Caz			BWC0977	Doxy/Caz
<i>B. anthracis</i>	Ames 3898	0.015	0.031	<i>B. pseudomallei</i>	1026b	0.25	4.00	<i>F. tularensis</i>	Schu4 S4 SCB	<0.007	1.00
<i>B. anthracis</i>	Graves	0.015	0.031	<i>B. pseudomallei</i>	K96243	1.00	4.00	<i>F. tularensis</i>	Schu4 S4	<0.007	1.00
<i>B. anthracis</i>	46-PY-5	0.015	0.031	<i>B. pseudomallei</i>	DD503	0.25	4.00	<i>F. tularensis</i>	WY96-3418	<0.007	1.00
<i>B. anthracis</i>	Ames	0.015	0.031	<i>B. pseudomallei</i>	BP400	0.02	2.00	<i>F. tularensis</i>	MA00-2987	<0.007	1.00
<i>B. anthracis</i>	Kruger B (A0442)	<0.007	0.016	<i>B. pseudomallei</i>	1106b	0.50	4.00	<i>F. tularensis</i>	KY99-3387	<0.007	1.00
<i>B. anthracis</i>	Vollum (A0488)	<0.007	0.031	<i>B. pseudomallei</i>	406e	1.00	2.00	<i>F. tularensis</i>	OR96-0246	<0.007	1.00
<i>B. anthracis</i>	WNA	0.015	0.031	<i>B. pseudomallei</i>	MSHR435	0.50	8.00	<i>Y. pestis</i>	CO92	<0.007	1.00
<i>B. anthracis</i>	A0471	0.015	0.031	<i>B. pseudomallei</i>	NCTC 6700	0.25	4.00	<i>Y. pestis</i>	ZE94-2122	<0.007	2.00
<i>B. anthracis</i>	ASC506	0.015	0.031	<i>B. pseudomallei</i>	NCTC 7383	4.00	4.00	<i>Y. pestis</i>	PEXU2	<0.007	2.00
<i>B. anthracis</i>	ASC525	0.015	0.031	<i>B. pseudomallei</i>	NCTC 10274	0.50	2.00	<i>Y. pestis</i>	PB6	<0.007	1.00
<i>B. anthracis</i>	ASC32	0.015	0.031	<i>B. mallei</i>	ATCC23344	1.00	2.00	<i>Y. pestis</i>	Nepal516	<0.007	1.00
<i>B. anthracis</i>	ASC149	0.015	0.031	<i>B. mallei</i>	China 7 NBL7	2.00	2.00	<i>B. mallei</i>	NCTC 10260	4.00	4.00
<i>B. mallei</i>	NCTC 120	0.50	4.00	<i>B. mallei</i>	GB8 H4	2.00	4.00	<i>B. mallei</i>	NCTC 12938	1.00	2.00
<i>B. mallei</i>	NCTC 10248	0.50	2.00								

<i>P. aeruginosa</i> , Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
ATCC 27853, CLSI control	1	0.5
AR 231 [KPC-5,OXA-50,PAO,OXA-2]	>64	0.5
AR 235 [VEB-1,OXA-50,PAO,OXA-10]	32	1
AR 244, 258, 263 [OXA-50]	16, 0.5, 0.5	0.5 - 1.0
AR 259, 262, 238, 253, 251, 261 [OXA-50,PAO]	0.5 - 1.0	0.5 - 2.0
AR 234, 237, 247, 272, 232, 256, 257, 267, 269, 270, 243, 248, 265, 271 [OXA-50,PAO]	2.0 - 8.0	0.125 - 0.5
AR 264 [OXA-50, PAO]	4	8
AR 233, 236, 245, 260, 266, 268, 229 [OXA-50,PAO]	16 - 32	0.125 - 2
AR 246, 250, 249 [OXA-50,PAO]	>64	0.5
AR 230, 254, 255 [VIM-2,OXA-50,PAO,OXA-4]	>64	0.25
AR 239 [VIM-11,OXA-50,OXA-10,GES-1]	64	0.5

<i>A. baumannii</i> , Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
AR 276	>64	1
AR 300 [ADC-25,OXA-66]	4	0.5
AR 303, 273, 288, 297 [ADC-25,OXA-23,OXA-66]	32 - 64	0.25 - 0.5
AR 311 [ADC-25,OXA-23,OXA-82]	>64	0.125
AR 286 [ADC-25,OXA-66,OXA-24]	>64	0.25
AR 304, 301, 287, 289 [ADC-25,OXA-66,OXA-72]	64 - >64	0.125 - 0.25
AR 296 [ADC-25,OXA-23,OXA-223]	64	0.25
AR 307 [ADC-25,OXA-66,OXA-237]	16	0.25
AR 312 [OXA-69]	8	2
AR 292, 293 [OXA-66,OXA-72]	>64	0.125 - 0.25
AR 302, 309, 310 [OXA-23,OXA-82]	>64	0.5 - 1
AR 299 [PER-7,OXA-23,OXA-203]	64	0.5
AR 277, 284, 285, 305, 306 [TEM-1B,OXA-65,OXA-24]	>64	0.5 - 2.0
AR 294 [TEM-1B,OXA-23,OXA-65]	64	0.25
AR 280 [TEM-1D,ADC-25,OXA-66]	2	0.5
AR 281 [TEM-1D,ADC-25,OXA-82]	16	0.125
AR 308 [TEM-1D,ADC-25,OXA-71]	16	0.5
AR 313 [TEM-1D,OXA-23,OXA-69]	32	0.125
AR 275, 279, 282, 291, 283, 278, 290, 295 [TEM-1D,ADC-25, OXA-23,OXA-66]	64	0.25 - 0.5
AR 274 [TEM-1D,ADC-25,OXA-66,OXA-72]	>64	0.5
AR 298 [TEM-1D,ADC-25,OXA-66,OXA-237]	32	0.5

Other Enterobacteriaceae, Isolate #, Phenotype / Beta-lactamases	MIC (µg/ml) Meropenem	MIC (µg/ml) BWC0977
Enterobacter cloacae AR 164, NMC-A	32	0.25
Kluyvera ascorbata AR 144, KPC-3,TEM-1B,CTX-M-124	8	4
Morganella morganii AR 133, KPC-2	4	2
Proteus mirabilis AR 155, KPC-6	4	1
Proteus mirabilis AR 156, KPC-2,OXA-10	0.5	0.125
Proteus mirabilis AR 159, NDM-1	8	0.06
S. marcescens AR 121, 122, 123, 124, 130, 131, SME-3	16 - 64	0.25 - 2.0