

Supporting Information

Neisseria gonorrhoeae becomes susceptible to polymyxin B and colistin in the presence of PBT2.

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Table S1. A study to establish the sub-inhibitory concentrations of PBT2/zinc so that the potential for PBT2/zinc to synergize with other antibiotics could be tested.

	PBT2 (μM)											
	0	0.01	0.02	0.05	0.1	0.25	0.5	1	2	4	8	16
ZnSO ₄ (μM)	0											
	1.25											
	2.5						selected					
	5											
	10											
	20											
	40											

“Selected” indicates the concentration of PBT2/zinc (PBT2 (0.5 μM)/zinc (2.5 μM)) that was 4-fold less than the MIC of PBT2 or PBT2/zinc. MIC of ZnSO₄ was not determined.

Table S2. MICs of synergistic antimicrobial activity of PBT2/zinc and cefixime, ceftriaxone, azithromycin and ciprofloxacin with WHO Z strain.

Antibiotics (mg/L)	BT2: 0 μM Zinc: 0 μM	PBT2: 1 μM Zinc: 5 μM	PBT2: 1 μM	Zinc: 5 μM
Cefixime	2 (R)	1 (R)	2 (R)	1 (R)
Ceftriaxone	2 (R)	1 (R)	2 (R)	1 (R)
Azithromycin	1 (R)	1 (R)	1 (R)	1 (R)
Ciprofloxacin	>50 (R)	50 (R)	50 (R)	50 (R)
Kanamycin	8 (S)	4 (S)		
Gentamicin	8 (S)	2 (S)		

Table S3. The relative abundance of the lipid A ions observed in MALDI. The PE reduced in the lipid A purified from WT growing in PBT2/zinc is $49.52 \pm 1.97\%$ (statistical significance between WT treated vs untreated PBT2/zinc was calculated using student T-test, $P=0.0094$).

Sample	Mass	Molecule	Abund.	Abd.[%]	Std.Dev.	Reduction[%]
WT						
A	1696.029	[M-H] ⁻	57021	100	0.085	
B	1616.071	[M-H] ⁻	19087	33.47	0.319	
C	1739.072	[M-H] ⁻	52291	91.7	0.067	
D	1819.056	[M-H] ⁻	47390	83.11	0.063	
LptA						
A	1696.053	[M-H] ⁻	286881	100	0.035	
B	1616.099	[M-H] ⁻	88041	30.69	0.043	
C				0		
D				0		
WT in PBT2/zinc						
A	1695.999	[M-H] ⁻	279496	100	0.067	
B	1616.043	[M-H] ⁻	74109	26.52	0.037	
C	1739.043	[M-H] ⁻	121859	43.6	0.039	47.55 ± 1.97
D	1819.026	[M-H] ⁻	119596	42.79	0.088	51.49 ± 1.97

Table S4. The integrals of the HSQC signals of phosphoethanoline of lipid A observed in NMR to quantitate the impact of the LptA mutation and treatment with PBT2/zinc on *N. gonorrhoeae* WHO Z. The PE reduced in the lipid A purified from WT growing in PBT2/zinc is $46.43 \pm 4.08\%$ (statistical significance between WT treated vs untreated PBT2/zinc was calculated using student T-test, $P=0.0139$).

Sample	Integral[ref]	F2[ppm]	F1[ppm]	Reduction[%]
WT				
ref.	1	3.3	48.1	
C1	0.0695	3.2	39.8	
C2	0.0789	4.2	62.2	
LptA				
ref.	1	3.3	48.52	
C1	-	-	-	
C2	-	-	-	
WT in PBT2/Zn				
ref.	1	3.3	48.5	
C1	0.0350	3.2	39.9	42.35 ± 4.08
C2	0.0338	4.2	61.8	50.50 ± 4.08

Figure S1. Time-kill curves of *N. gonorrhoeae* WHO Z in GC broth with or without PBT2/zinc (PBT2 (0.5 μ M)/zinc (2.5 μ M)), in the absence and presence of colistin (1.56 mg/L) or polymyxin B (0.98 mg/L). Error bars indicate standard deviation from 3 biological replicates.

