

Supplementary Materials: A New Surface Charge Neutralizing Nano-Adjuvant to Potentiate Polymyxins in Killing Mcr-1 Mediated Drug-Resistant *Escherichia coli*

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Table S1. Sequence of synthesized mobilized colistin resistance gene (*mcr-1*). The sequences of *mcr-1* were taken from NCBI protein database (GenBank ID: ASK04346.1) and further codon optimized by GenSmart™ Codon Optimization (ver. Beta 1.0; <https://www.genscript.com/tools/gensmart-codon-optimization>; GenScript, Piscataway, NJ, USA). Red sequences indicate the modified DNA sequences by codon optimization.

Gene	DNA Sequence (5' → 3')
<i>mcr-1</i> (1627 bp)	ATGATGCAGCACACCAGCGTTGGTATCGTCGTAGCGTTAGCCCGTTCGTTCTGGTTGCG AGCGTTGCGGTTTTCTGACCGCGAACCTGACCTTCCTTGACAAGATCAGCCAA ACCTACCGGATTGCGGATAACCTGGGTTTCGTTCTGACCATCGCGGTGGTTCTGTTTGGC GCGATGCTGCTGATTACACCCCTGCTGAGCAGCTACCGTTATGTGCTGAAACCGGTTCTG ATCTGCTGCTGATTATGGGTGCGGTTACCGTACCTTACCGACACCTACGGCACCGTG TATGATACCAACATGCTGCAGAACGCGCTGCAGACCGACCAAGCGAAACCAAGGATCTG CTGAACGCGGCGTTTCATTATGCGTATCATTGGTCTGGGCGTGCTGCCGAGCCTGCTGGTT GCGTTTGTGAAGTTGACTACCGACCTGGGGTAAAGGCTGATGCGTCGTCTGGGTCTG ATCGTGGCGAGCCTGGCGCTGATTCTGCTGCCGCTGTTGGCTTCAGCAGCCACTATGCG AGCTTCTTTCGTGTGCACAAGCCGCTGCGTAGCTACGTTAACCCGATCATGCCGATTAT AGCGTTGGCAAACTGGCGAGCATCGAATACAAGAAAGCGAGCGCGCCGAAGGACACCATT TATCACGCGAAAGATGCGGTGCAAGCGACCAAGCCGACATGCGTAAACCGCGTCTGGTG GTTTTTGTGGTTGGTGAAACCGCGCGTGGGATCACGTTAGCTTCAACGGCTACGAACGT GACACCTTTCCGAGCTGGCGAAGATCGATGGTGTGACCAACTTCAGCAACGTTACCAGC TGCGGTACCAGCACCGCGTACAGCGTGCCGTGCATGTTTAGCTACCTGGGTGCGGATGAG TATGACGTGGATACCGCGAAATATCAAGAAACGTTCTGGACACCCCTGGATCGTCTGGT GTGAGCATCCTGTGGCGTGACAACAACAGCGATAGCAAGGGCGTTATGGACAAGCTGCCG AAAGCGCAGTTCGCGGATTACAAAAGCGCGACCAACACCGGATTGCAACACCAACCCG TATAACGAGTGCCCGTGACGTGGGTATGCTGTTGGCCTGGACGATTTCGTTGCGGCGAAC AACGGCAAGGATATGCTGATCATGCTGCACCAATGGGTAAACACGGCCCGCGGTACTTT AAACGTTATGACGAGAAGTTTCGCGAAATTTACCCCGGTGTGCGAGGGCAACGAACCTGGCG AAATGCGAACACCAGAGCCTGATCAACGCGTACGATAACGCGCTGCTGGCGACCGACGAT TTCATCGCGCAGAGCATTCAATGGCTGCAGACCCACAGCAACGCGTACGACGTGAGCATG CTGTATGTTAGCGATCACGGCGAGAGCCTGGGTGAAAACGGCGTTTATCTGCACGGCATG CCGAACGCGTTTGGCGCGAAGGAACAACGTAGCGTGCCGGCGTTCTTTGGACCGACAAA CAGACCGGTATCACCCCGATGGCGACCGACACCGTGCTGACCCACGATGCGATTACCCCG ACCTGCTGAAGCTGTTGATGTTACCGCGGACAAAGGTGAAGACCGTACCGCGTTTATT CGT

Table S2. Strains and plasmids used in this study.

Strains		
Name	Feature	Reference ¹
BW25113	<i>F</i> Δ (<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787:: <i>rrnB</i> -3, <i>LAM</i> ⁻ <i>rph</i> -1	[1]
Keio- <i>arnT</i>	Δ (<i>rhaD-rhaB</i>)568 <i>hsdR</i> 514	[2]
Keio- <i>eptA</i>	BW25113 Δ <i>eptA</i> :: <i>kan</i> ^R	[2]
KS7000	BW25113 pQE60	This study
KS8000	BW25113 pQE60- <i>mcr</i> -1	This study
ATCC19606	<i>Acinetobacter baumannii</i> Bouvet and Grimont	American Type Culture Collection (ATCC) (www.atcc.org)
ATCC27853	<i>Pseudomonas aeruginosa</i> (Schroeter) Mugula	ATCC
Clinical isolates		
NCCP16283	<i>Escherichia coli</i> , Colistin ^R , <i>mcr</i> -1	National Culture Collections for Pathogens (NCCP)
NCCP16284	<i>Escherichia coli</i> , Colistin ^R , <i>mcr</i> -1, <i>bla</i> _{NDM-1} , ESBLs	NCCP
NCCP16285	<i>Klebsiella pneumoniae</i> , Colistin ^R , <i>mcr</i> -1, <i>bla</i> _{NDM-1} , ESBLs	NCCP
BAA-2340	Carbapenems ^R , <i>bla</i> _{NDM-1} / <i>bla</i> _{KPC} +	ATCC
BAA-2471	Carbapenems ^R , <i>bla</i> _{NDM-1} / <i>bla</i> _{KPC} -	ATCC
Plasmids		
pQE60	Ampicillin	Qiagen
pQE60- <i>mcr</i> -1	Colistin, polymyxin B, Ampicillin	This study
Primer sequence (5' → 3')		
pQE60-F	CCC GAA AAG TGC CAC CTG	
pQE60-R	GTT CTG AGG TCA TTA CTG G	

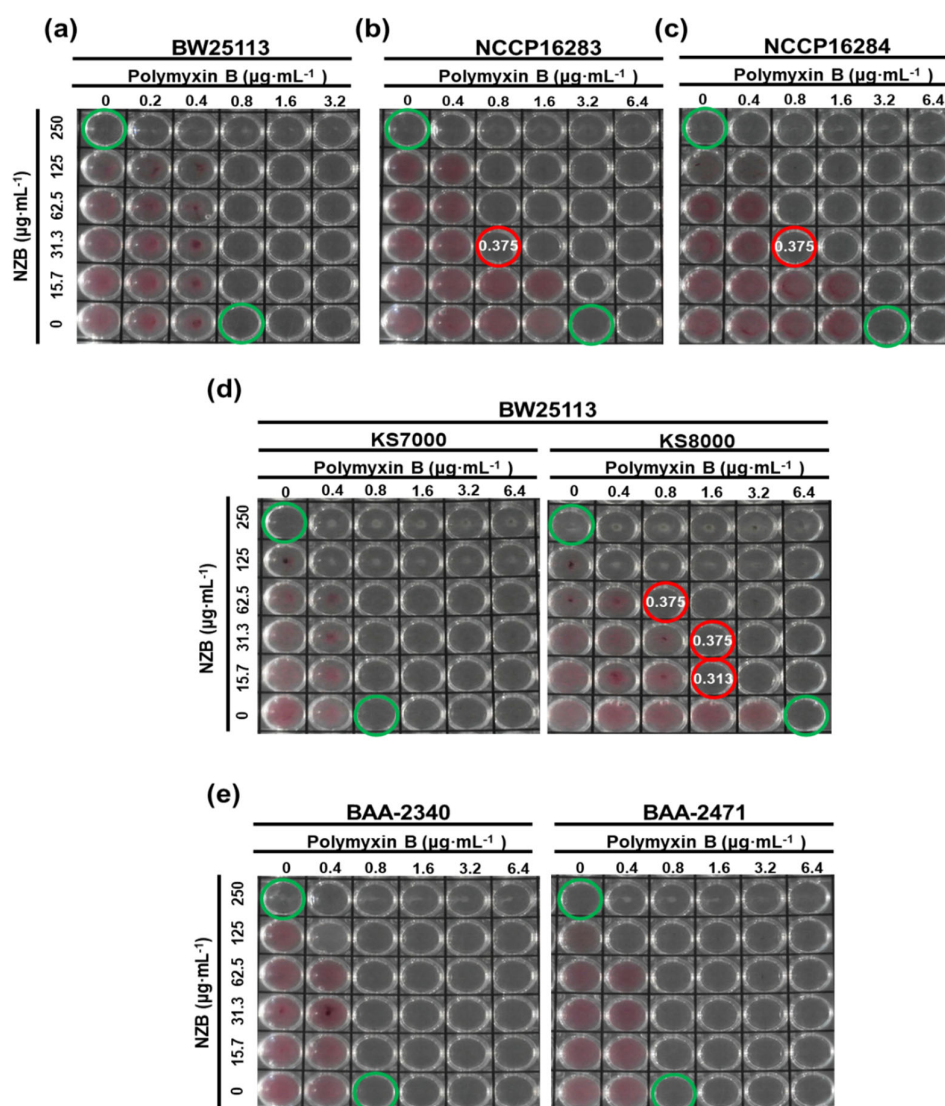


Figure S1. Synergy activity of NZB to polymyxin B (PolB) against *E. coli* cells. Checkerboard assays for (a) BW25113 and (b)–(d) *mcr-1* harboring *E. coli* cells either plasmid incorporated (pQE60 or pQE60-*mcr-1* in BW25113, i.e. KS7000 or KS8000, respectively) or clinical isolates (NCCP16283 and 16284), (e) MDR *E. coli* cells (BAA-2340 and -2471) were shown. The MIC point of NZB and PolB was shown in green circle. Synergistic positions (FICI < 0.5) were indicated with red circles. One of the representatives from $n = 3$ was shown. FICI values were indicated in Table 1. The strain information is available in Table S1.

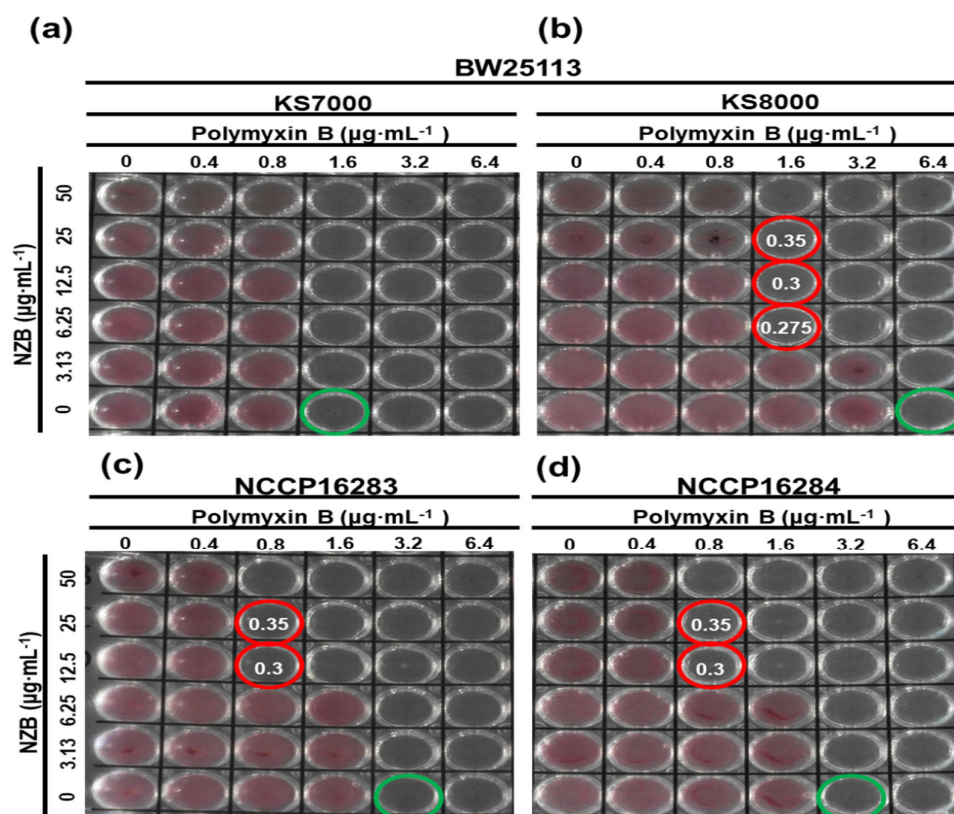


Figure S2. Synergistic activity of NZB to polymyxin B (PolB) against Mcr-1 expressing *E. coli* cells. Checkerboard assays results for (a) KS7000 (pQE60 in BW25113; non-*mcr-1*), (b) KS8000 (pQE60-*mcr-1* in BW25113), (c) NCCP16283, and (d) NCCP16284 were shown. The MIC point of PolB was shown in green circle. Synergistic positions (FICI < 0.5) were indicated with red circles. One of the representatives from $n = 3$ was shown. The stain information is available in Table S2. FICI values were shown in Table 1.

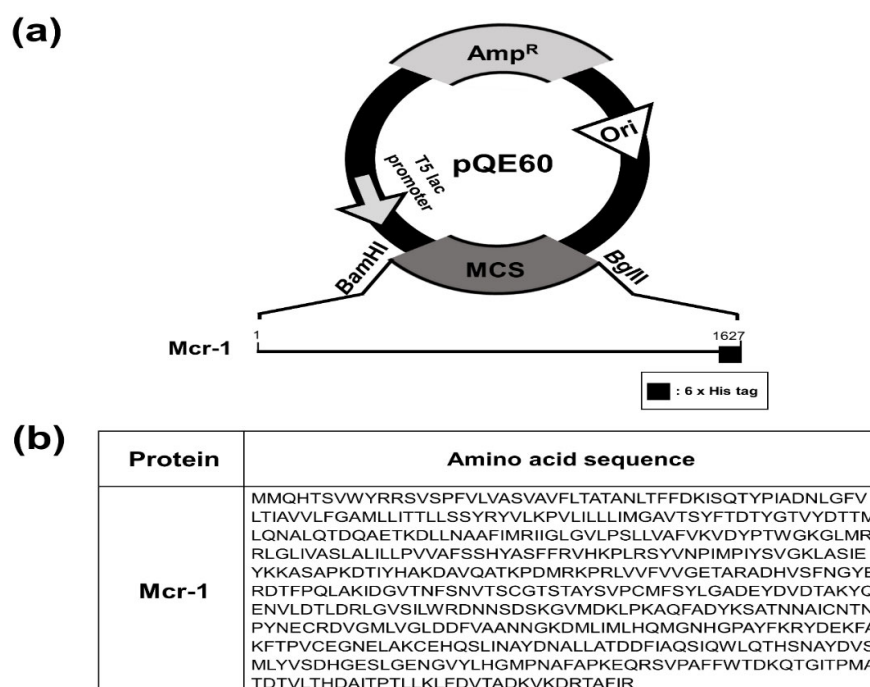


Figure S3. Features of Mcr-1 protein expression plasmid (pQE60-*mcr-1*). (a) Schematic representation of Mcr-1 protein coding regions with C-terminal histidine tag in pQE60 vector and (b) amino acid sequences of Mcr-1.

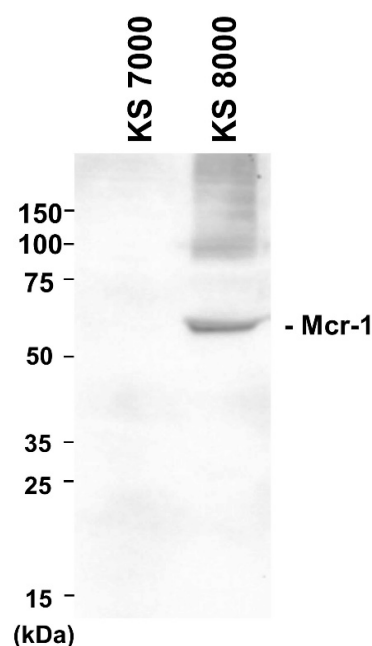


Figure S4. Expression of Mcr-1 protein. Mcr-1 expression from KS7000 (pQE60) and KS8000 (pQE60-*mcr-1*) strains was detected by western blotting with antibodies against His-tag. Image acquisition and the quantitative analysis were performed by using the ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA) and Image Lab (ver 5.2.1; Bio-Rad, Hercules, CA, USA).

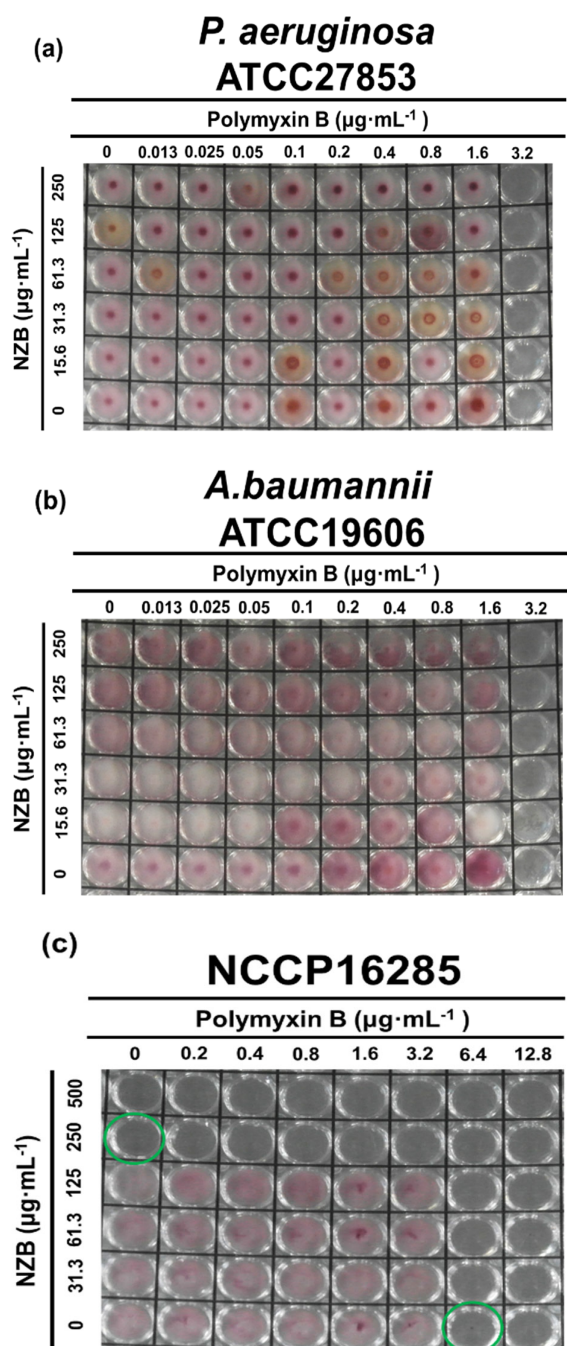


Figure S5. Synergistic activity of NZB to polymyxin B (PolB) against non-*E. coli* Gram-negative strains. Checkerboard assays results for (a) ATCC27853 (*P. aeruginosa*), (b) ATCC19606 (*A. baumannii*), and (c) NCCP16285 (*Klebsiella pneumoniae mcr-1* clinical isolates) were shown. One of the representatives from $n = 3$ was shown. The MIC point of PolB was shown in green circle. Synergistic positions (FICI < 0.5) were indicated with red circles. FICI values were indicated in Table 1.

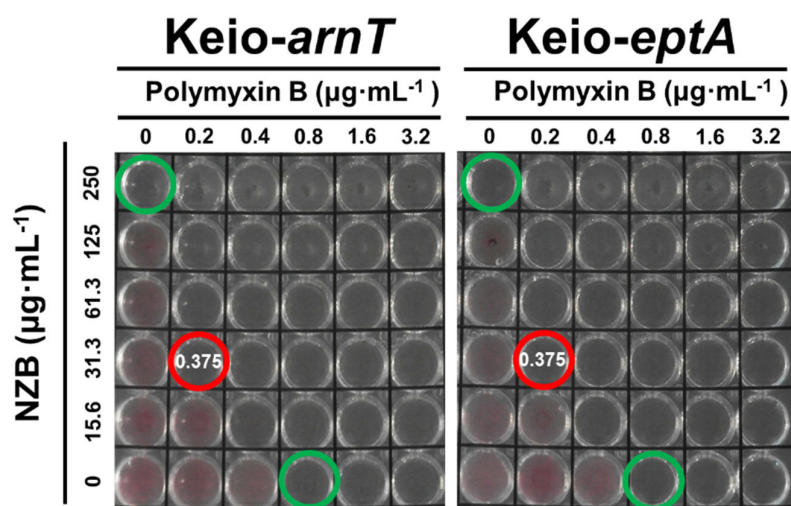


Figure S6. Evaluation of synergistic activity of Mcr-1 modified gene knockouts. Checkerboard assays for NZB and polymyxin B (PolB) against Keio-*arnT* (left) and Keio-*eptA* (right) were shown. One of the representatives from $n = 3$ was shown. The MIC point of PolB was shown in green circle. Synergistic positions ($FICI < 0.5$) were indicated with red circles. FICI values were shown in Table 1.

References

1. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA*. **2000**, *97*, 6640–6645.
2. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K.A.; Tomita, M.; Wanner, B.L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006**, *2*, 2006.0008.