

## Supplementary data

### **Bacterial Cytological Profiling (BCP) as a tool to study mechanism of action of antibiotics that are active against *Acinetobacter baumannii***

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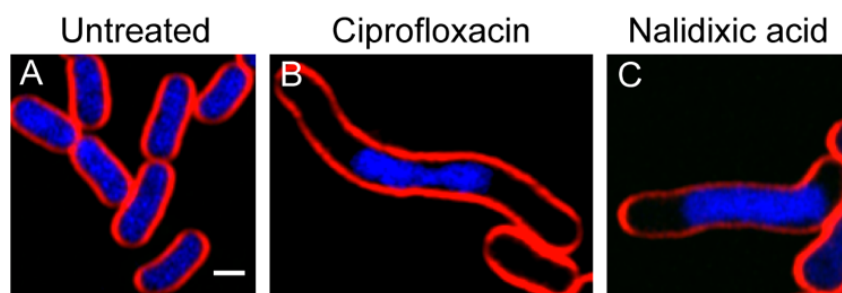
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Running title: BCP for *A. baumannii* antibiotic research

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27 **FIG S1 Cytological profile of DNA synthesis inhibitors in *A. baumannii* ATCC 19606**  
28 **cells.**

29 (A) Untreated cells. Bacterial cells were treated with 2x MIC of (B) ciprofloxacin and (C)  
30 nalidixic acid for 2 hours and then stained with FM4-64 (red) and DAPI (blue). Scale bar  
31 represents 1  $\mu\text{m}$ .

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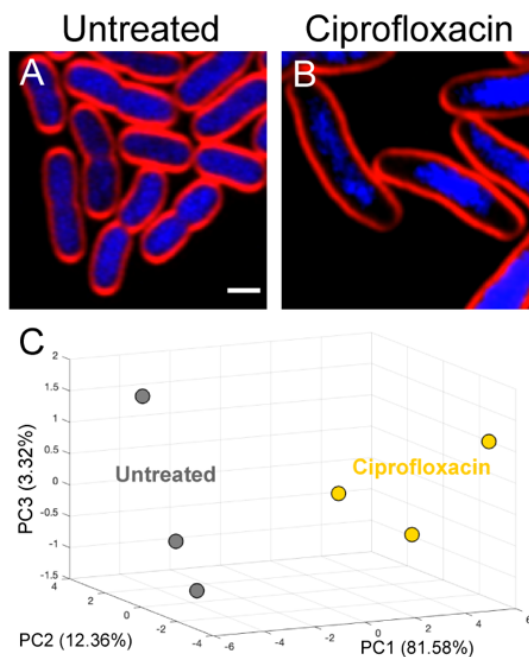
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47 **FIG S2 Cytological profiling of DNA synthesis inhibitor, ciprofloxacin, in *A. baumannii***

48 **ATCC 17978 cells.**

49 (A) Untreated cells, scale bar represents 1  $\mu\text{m}$ . (B) Bacterial cells were treated with 2x MIC of

50 ciprofloxacin for 2 hours and then stained with FM4-64 (red) and DAPI (blue). (C) PCA graph

51 of untreated and ciprofloxacin-treated *A. baumannii* ATCC 17978 cells, made by using PC1

52 (81.58%), PC2 (12.36%) and PC3 (3.32%), showing Ciprofloxacin-treated cells in distinct

53 group from untreated cells.

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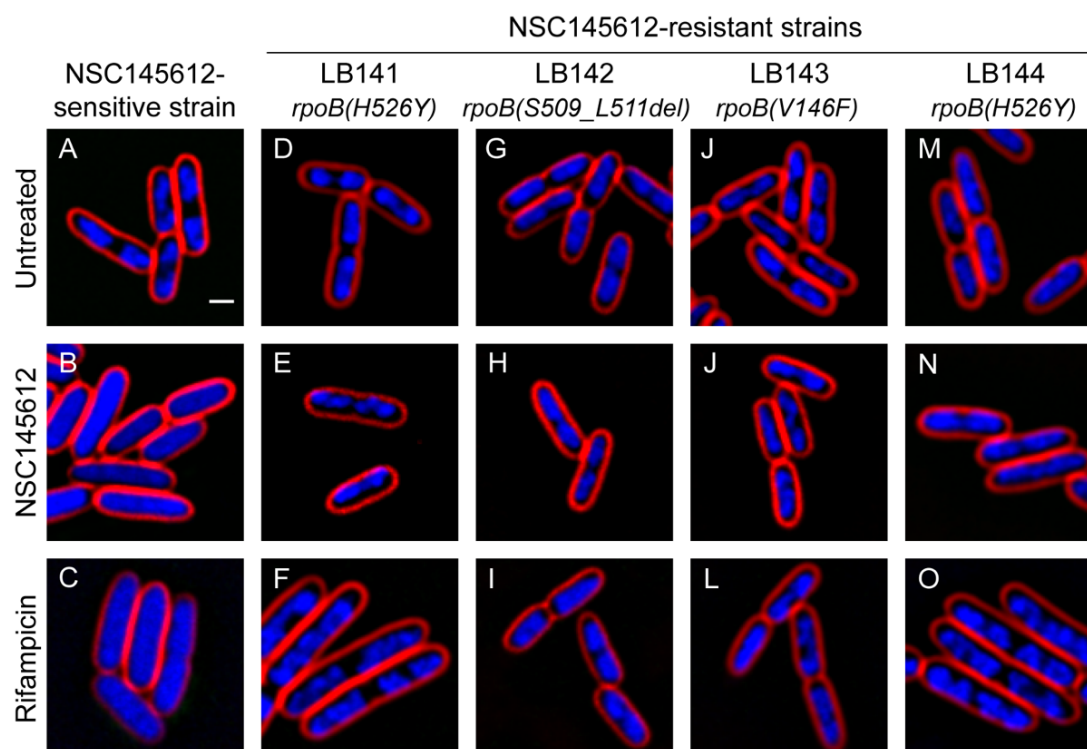
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65 **FIG S3 NSC145612-sensitive and -resistant *E. coli* cells treated with NSC145612 and**66 **Rifampicin**67 (*A–C*) NSC145612-sensitive strain. (*D–O*) NSC145612-resistant strains with *rpoB* mutations

68 indicated. Bacterial cells were treated with NSC145612 or Rifampicin for 2 hours and then

69 stained with FM4-64 (red) and DAPI (blue). Scale bar represents 1  $\mu\text{m}$ .

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79 **Table S1** Complete list of drugs tested in this study

Antibiotic Class	Antibiotic Name	MIC ( $\mu\text{g/ml}$ )	Target
<b>Drugs tested in <i>Acinetobacter baumannii</i> ATCC 19606</b>			
<b>Protein Synthesis Inhibitors</b>			
Aminoglycoside	Amikacin	20	30s ribosome (promote mistranslation)
	Gentamicin	28	30s ribosome (promote mistranslation)
	Kanamycin	10	30s ribosome (promote mistranslation)
	Streptomycin	>112	30s ribosome (promote mistranslation)
	Tobramycin	7	30s ribosome (inhibit initiation complex formation)
Amphenicols	Chloramphenicol	50	50s ribosome (inhibit peptidyl transferase)
Macrolide	Azithromycin	16	50S ribosome (interfere aminoacyl translocation)
Tetracycline	Minocycline	0.75	30S ribosome (inhibit aminoacyl tRNA binding)
	Tetracycline	0.25	30S ribosome (inhibit aminoacyl tRNA binding)
	Tigecycline	2	30S ribosome (inhibit aminoacyl tRNA binding)
<b>RNA Transcription Inhibitor</b>			
Rifamycin	Rifampicin	1	DNA-dependent RNA polymerase
<b>Cell Wall Synthesis Inhibitors</b>			
Penicillin	Ampicillin	>112	Penicillin-binding proteins (PBPs)
	Amoxicillin	>112	Penicillin-binding proteins (PBPs)
	Mecillinam	>112	Penicillin-binding proteins (PBPs)
	Piperacillin	16	Penicillin-binding proteins (PBPs)
Carbapenem	Meropenem	1	Penicillin-binding proteins (PBPs)
Others	Fosfomycin	>112	UDP-N-acetylglucosamine enolpyruvyl transferase (MurA)
	D-Cycloserine	>112	D-Ala-D-Ala terminal of peptidoglycan
<b>Membrane Active Compounds</b>			
Polymyxin	Colistin	1	Lipopolysaccharide (LPS)
Oxidative Phosphorylation uncoupling Agents	2,4-Dinitrophenol	>112	Energy poisoning agents
<b>Lipid Synthesis Inhibitors</b>			
Polychloro Phenoxy Phenols	Triclosan	0.2	Bacterial enoyl-acyl carrier protein reductase enzyme (ENR: FabI product)
<b>Tested compound</b>	NSC145612	25 $\mu\text{M}$	
<b>Drugs tested in <i>Acinetobacter baumannii</i> ATCC 17978</b>			
<b>DNA Synthesis Inhibitor</b>			
Fluoroquinolone	Ciprofloxacin	1	DNA gyrase A

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82 **Table S2** *A. baumannii* cell morphological measurements used in cytological profiling. All  
 83 values are in mean  $\pm$  SEM for 3 independent experiments.

Antibiotics	Membrane length, $\mu\text{m}$	DNA length, $\mu\text{m}$	Membrane width, $\mu\text{m}$	DNA width, $\mu\text{m}$
Amikacin	2.568 $\pm$ 0.023	2.378 $\pm$ 0.031	1.380 $\pm$ 0.000	1.182 $\pm$ 0.005
Azithromycin	2.368 $\pm$ 0.116	1.726 $\pm$ 0.020	0.922 $\pm$ 0.056	1.009 $\pm$ 0.025
Chloramphenicol	2.402 $\pm$ 0.033	1.892 $\pm$ 0.025	0.917 $\pm$ 0.029	1.068 $\pm$ 0.011
Colistin	2.107 $\pm$ 0.077	2.164 $\pm$ 0.175	1.039 $\pm$ 0.001	1.043 $\pm$ 0.046
Gentamicin	2.606 $\pm$ 0.033	2.520 $\pm$ 0.037	1.251 $\pm$ 0.009	1.168 $\pm$ 0.014
Kanamycin	2.572 $\pm$ 0.042	2.401 $\pm$ 0.041	1.325 $\pm$ 0.035	1.142 $\pm$ 0.023
Meropenem	3.596 $\pm$ 0.149	3.578 $\pm$ 0.149	2.626 $\pm$ 0.040	2.577 $\pm$ 0.038
Minocycline	2.392 $\pm$ 0.131	2.179 $\pm$ 0.082	0.961 $\pm$ 0.032	1.120 $\pm$ 0.012
Piperacillin	14.034 $\pm$ 1.005	13.809 $\pm$ 0.950	4.985 $\pm$ 0.988	4.878 $\pm$ 0.937
Rifampicin	2.680 $\pm$ 0.012	2.361 $\pm$ 0.077	1.066 $\pm$ 0.020	1.123 $\pm$ 0.029
Tetracycline	2.816 $\pm$ 0.056	2.006 $\pm$ 0.015	1.175 $\pm$ 0.012	1.155 $\pm$ 0.022
Tigecycline	2.667 $\pm$ 0.114	2.104 $\pm$ 0.078	1.122 $\pm$ 0.069	1.237 $\pm$ 0.034
Tobramycin	2.648 $\pm$ 0.058	2.548 $\pm$ 0.059	1.230 $\pm$ 0.033	1.145 $\pm$ 0.021
Triclosan	2.807 $\pm$ 0.079	2.742 $\pm$ 0.083	1.261 $\pm$ 0.031	1.216 $\pm$ 0.026
NSC145612	2.889 $\pm$ 0.057	2.469 $\pm$ 0.060	1.101 $\pm$ 0.019	1.150 $\pm$ 0.019
Untreated	2.962 $\pm$ 0.053	2.953 $\pm$ 0.023	0.987 $\pm$ 0.041	1.084 $\pm$ 0.055
Ciprofloxacin*	4.776 $\pm$ 0.177	4.041 $\pm$ 0.097	1.050 $\pm$ 0.015	1.040 $\pm$ 0.026
Untreated*	2.583 $\pm$ 0.024	2.639 $\pm$ 0.053	0.819 $\pm$ 0.013	0.934 $\pm$ 0.020

Antibiotics	Membrane area, $\mu\text{m}^2$	DNA area, $\mu\text{m}^2$	Membrane perimeter, $\mu\text{m}$	DNA perimeter, $\mu\text{m}$
Amikacin	2.743 $\pm$ 0.023	2.175 $\pm$ 0.037	7.002 $\pm$ 0.056	6.118 $\pm$ 0.061
Azithromycin	1.552 $\pm$ 0.168	1.378 $\pm$ 0.042	6.879 $\pm$ 0.753	4.857 $\pm$ 0.103
Chloramphenicol	1.602 $\pm$ 0.022	1.557 $\pm$ 0.041	6.256 $\pm$ 0.125	5.173 $\pm$ 0.068
Colistin	1.618 $\pm$ 0.054	1.703 $\pm$ 0.196	5.567 $\pm$ 0.137	7.169 $\pm$ 0.296
Gentamicin	2.496 $\pm$ 0.050	2.263 $\pm$ 0.061	6.958 $\pm$ 0.060	6.405 $\pm$ 0.078
Kanamycin	2.636 $\pm$ 0.101	2.116 $\pm$ 0.065	6.952 $\pm$ 0.137	6.142 $\pm$ 0.125
Meropenem	7.616 $\pm$ 0.460	7.431 $\pm$ 0.454	11.866 $\pm$ 0.645	12.075 $\pm$ 0.666
Minocycline	1.658 $\pm$ 0.134	1.851 $\pm$ 0.073	6.447 $\pm$ 0.374	5.948 $\pm$ 0.142
Piperacillin	26.189 $\pm$ 9.390	27.101 $\pm$ 10.523	70.243 $\pm$ 24.863	62.659 $\pm$ 18.868
Rifampicin	2.117 $\pm$ 0.049	2.076 $\pm$ 0.116	6.637 $\pm$ 0.072	6.671 $\pm$ 0.148
Tetracycline	2.447 $\pm$ 0.077	1.753 $\pm$ 0.041	8.112 $\pm$ 0.503	5.839 $\pm$ 0.133
Tigecycline	2.235 $\pm$ 0.214	2.037 $\pm$ 0.142	7.645 $\pm$ 0.280	6.195 $\pm$ 0.130
Tobramycin	2.493 $\pm$ 0.124	2.240 $\pm$ 0.094	6.972 $\pm$ 0.179	6.393 $\pm$ 0.141
Triclosan	2.627 $\pm$ 0.119	2.457 $\pm$ 0.114	7.788 $\pm$ 0.154	7.626 $\pm$ 0.099
NSC145612	2.353 $\pm$ 0.049	2.216 $\pm$ 0.079	7.186 $\pm$ 0.150	7.061 $\pm$ 0.167
Untreated	2.106 $\pm$ 0.154	2.390 $\pm$ 0.168	7.379 $\pm$ 0.279	7.451 $\pm$ 0.150
Ciprofloxacin*	3.219 $\pm$ 0.100	3.102 $\pm$ 0.137	11.457 $\pm$ 0.295	9.727 $\pm$ 0.185
Untreated*	1.500 $\pm$ 0.015	1.863 $\pm$ 0.079	6.247 $\pm$ 0.070	6.351 $\pm$ 0.117

Antibiotics	Membrane form factor	DNA form factor	Membrane compactness, $\mu\text{m}$	DNA compactness, $\mu\text{m}$
Amikacin	0.711 $\pm$ 0.005	0.732 $\pm$ 0.003	0.060 $\pm$ 0.000	0.063 $\pm$ 0.000
Azithromycin	0.468 $\pm$ 0.054	0.706 $\pm$ 0.008	0.082 $\pm$ 0.001	0.060 $\pm$ 0.000
Chloramphenicol	0.536 $\pm$ 0.013	0.729 $\pm$ 0.013	0.082 $\pm$ 0.002	0.061 $\pm$ 0.001
Colistin	0.682 $\pm$ 0.010	0.418 $\pm$ 0.037	0.067 $\pm$ 0.002	0.075 $\pm$ 0.002
Gentamicin	0.655 $\pm$ 0.005	0.695 $\pm$ 0.003	0.066 $\pm$ 0.000	0.067 $\pm$ 0.001
Kanamycin	0.688 $\pm$ 0.001	0.704 $\pm$ 0.006	0.062 $\pm$ 0.000	0.066 $\pm$ 0.000
Meropenem	0.721 $\pm$ 0.017	0.679 $\pm$ 0.020	0.053 $\pm$ 0.001	0.054 $\pm$ 0.001
Minocycline	0.526 $\pm$ 0.020	0.661 $\pm$ 0.008	0.079 $\pm$ 0.002	0.066 $\pm$ 0.001
Piperacillin	0.176 $\pm$ 0.016	0.165 $\pm$ 0.017	0.240 $\pm$ 0.027	0.243 $\pm$ 0.029
Rifampicin	0.621 $\pm$ 0.002	0.567 $\pm$ 0.011	0.076 $\pm$ 0.001	0.068 $\pm$ 0.000
Tetracycline	0.523 $\pm$ 0.035	0.635 $\pm$ 0.014	0.075 $\pm$ 0.001	0.065 $\pm$ 0.001
Tigecycline	0.487 $\pm$ 0.035	0.652 $\pm$ 0.016	0.076 $\pm$ 0.003	0.062 $\pm$ 0.000
Tobramycin	0.650 $\pm$ 0.002	0.689 $\pm$ 0.002	0.067 $\pm$ 0.000	0.069 $\pm$ 0.000
Triclosan	0.566 $\pm$ 0.005	0.563 $\pm$ 0.029	0.070 $\pm$ 0.001	0.072 $\pm$ 0.001
NSC145612	0.592 $\pm$ 0.012	0.536 $\pm$ 0.011	0.080 $\pm$ 0.002	0.070 $\pm$ 0.000
Untreated	0.509 $\pm$ 0.023	0.552 $\pm$ 0.010	0.091 $\pm$ 0.004	0.081 $\pm$ 0.003
Ciprofloxacin*	0.327 $\pm$ 0.009	0.429 $\pm$ 0.004	0.154 $\pm$ 0.007	0.111 $\pm$ 0.001
Untreated*	0.524 $\pm$ 0.004	0.594 $\pm$ 0.005	0.097 $\pm$ 0.002	0.082 $\pm$ 0.000

Antibiotics	Membrane extent, $\mu\text{m}$	DNA extent, $\mu\text{m}$	Membrane eccentricity, $\mu\text{m}$	DNA eccentricity, $\mu\text{m}$
Amikacin	0.032 $\pm$ 0.000	0.032 $\pm$ 0.000	0.039 $\pm$ 0.000	0.040 $\pm$ 0.000
Azithromycin	0.027 $\pm$ 0.001	0.032 $\pm$ 0.000	0.043 $\pm$ 0.000	0.036 $\pm$ 0.000
Chloramphenicol	0.027 $\pm$ 0.000	0.032 $\pm$ 0.000	0.043 $\pm$ 0.001	0.037 $\pm$ 0.000
Colistin	0.031 $\pm$ 0.000	0.027 $\pm$ 0.001	0.038 $\pm$ 0.001	0.039 $\pm$ 0.001
Gentamicin	0.031 $\pm$ 0.000	0.031 $\pm$ 0.000	0.040 $\pm$ 0.000	0.041 $\pm$ 0.000
Kanamycin	0.032 $\pm$ 0.000	0.032 $\pm$ 0.000	0.039 $\pm$ 0.000	0.040 $\pm$ 0.000
Meropenem	0.034 $\pm$ 0.000	0.034 $\pm$ 0.000	0.028 $\pm$ 0.001	0.029 $\pm$ 0.001
Minocycline	0.027 $\pm$ 0.001	0.031 $\pm$ 0.000	0.042 $\pm$ 0.000	0.038 $\pm$ 0.001
Piperacillin	0.013 $\pm$ 0.000	0.013 $\pm$ 0.000	0.044 $\pm$ 0.001	0.044 $\pm$ 0.001
Rifampicin	0.043 $\pm$ 0.000	0.041 $\pm$ 0.000	0.029 $\pm$ 0.000	0.030 $\pm$ 0.000
Tetracycline	0.028 $\pm$ 0.000	0.031 $\pm$ 0.000	0.042 $\pm$ 0.000	0.036 $\pm$ 0.000
Tigecycline	0.028 $\pm$ 0.001	0.032 $\pm$ 0.000	0.042 $\pm$ 0.001	0.036 $\pm$ 0.001
Tobramycin	0.031 $\pm$ 0.000	0.031 $\pm$ 0.000	0.041 $\pm$ 0.000	0.041 $\pm$ 0.000
Triclosan	0.029 $\pm$ 0.001	0.029 $\pm$ 0.001	0.041 $\pm$ 0.001	0.041 $\pm$ 0.001
NSC145612	0.028 $\pm$ 0.000	0.029 $\pm$ 0.000	0.043 $\pm$ 0.000	0.040 $\pm$ 0.000
Untreated	0.026 $\pm$ 0.001	0.027 $\pm$ 0.000	0.044 $\pm$ 0.000	0.044 $\pm$ 0.000
Ciprofloxacin*	0.018 $\pm$ 0.001	0.023 $\pm$ 0.000	0.046 $\pm$ 0.000	0.046 $\pm$ 0.000
Untreated*	0.026 $\pm$ 0.000	0.028 $\pm$ 0.000	0.045 $\pm$ 0.000	0.044 $\pm$ 0.000

Antibiotics	Membrane maximum ferret diameter, $\mu\text{m}$	DNA maximum ferret diameter, $\mu\text{m}$	Membrane minimum ferret diameter, $\mu\text{m}$	DNA minimum ferret diameter, $\mu\text{m}$
Amikacin	2.456 $\pm$ 0.021	2.247 $\pm$ 0.030	1.352 $\pm$ 0.002	1.147 $\pm$ 0.005
Azithromycin	2.206 $\pm$ 0.125	1.658 $\pm$ 0.018	0.940 $\pm$ 0.067	0.990 $\pm$ 0.027
Chloramphenicol	2.248 $\pm$ 0.012	1.801 $\pm$ 0.025	0.922 $\pm$ 0.034	1.046 $\pm$ 0.010
Colistin	1.970 $\pm$ 0.067	2.070 $\pm$ 0.157	1.028 $\pm$ 0.003	1.076 $\pm$ 0.047
Gentamicin	2.464 $\pm$ 0.028	2.362 $\pm$ 0.030	1.236 $\pm$ 0.007	1.141 $\pm$ 0.013
Kanamycin	2.453 $\pm$ 0.040	2.258 $\pm$ 0.039	1.302 $\pm$ 0.034	1.113 $\pm$ 0.023
Meropenem	3.548 $\pm$ 0.134	3.521 $\pm$ 0.133	2.632 $\pm$ 0.048	2.585 $\pm$ 0.046
Minocycline	2.262 $\pm$ 0.119	2.057 $\pm$ 0.071	0.966 $\pm$ 0.036	1.110 $\pm$ 0.014
Piperacillin	13.023 $\pm$ 1.188	12.914 $\pm$ 1.170	5.252 $\pm$ 1.154	5.191 $\pm$ 1.142
Rifampicin	2.469 $\pm$ 0.020	2.250 $\pm$ 0.069	1.052 $\pm$ 0.020	1.117 $\pm$ 0.027
Tetracycline	2.698 $\pm$ 0.045	1.909 $\pm$ 0.022	1.181 $\pm$ 0.013	1.150 $\pm$ 0.026
Tigecycline	2.523 $\pm$ 0.095	2.039 $\pm$ 0.076	1.150 $\pm$ 0.066	1.226 $\pm$ 0.029
Tobramycin	2.503 $\pm$ 0.057	2.386 $\pm$ 0.055	1.220 $\pm$ 0.030	1.122 $\pm$ 0.018
Triclosan	2.654 $\pm$ 0.062	2.580 $\pm$ 0.057	1.277 $\pm$ 0.032	1.230 $\pm$ 0.024
NSC145612	2.677 $\pm$ 0.046	2.367 $\pm$ 0.058	1.097 $\pm$ 0.023	1.144 $\pm$ 0.020
Untreated	2.731 $\pm$ 0.056	2.765 $\pm$ 0.035	1.004 $\pm$ 0.043	1.094 $\pm$ 0.056
Ciprofloxacin*	4.567 $\pm$ 0.188	3.821 $\pm$ 0.089	1.125 $\pm$ 0.013	1.071 $\pm$ 0.029
Untreated*	2.390 $\pm$ 0.017	2.456 $\pm$ 0.048	0.815 $\pm$ 0.012	0.921 $\pm$ 0.018

Antibiotics	Membrane maximum radius, $\mu\text{m}$	DNA maximum radius, $\mu\text{m}$	Membrane mean radius, $\mu\text{m}$	DNA mean radius, $\mu\text{m}$
Amikacin	0.656 $\pm$ 0.002	0.566 $\pm$ 0.003	0.268 $\pm$ 0.001	0.236 $\pm$ 0.002
Azithromycin	0.407 $\pm$ 0.020	0.473 $\pm$ 0.009	0.167 $\pm$ 0.008	0.192 $\pm$ 0.002
Chloramphenicol	0.424 $\pm$ 0.008	0.499 $\pm$ 0.008	0.178 $\pm$ 0.001	0.203 $\pm$ 0.003
Colistin	0.497 $\pm$ 0.002	0.429 $\pm$ 0.032	0.203 $\pm$ 0.001	0.169 $\pm$ 0.013
Gentamicin	0.587 $\pm$ 0.006	0.554 $\pm$ 0.008	0.242 $\pm$ 0.002	0.232 $\pm$ 0.003
Kanamycin	0.624 $\pm$ 0.015	0.542 $\pm$ 0.010	0.256 $\pm$ 0.005	0.227 $\pm$ 0.004
Meropenem	1.193 $\pm$ 0.007	1.138 $\pm$ 0.005	0.438 $\pm$ 0.002	0.418 $\pm$ 0.001
Minocycline	0.444 $\pm$ 0.014	0.519 $\pm$ 0.005	0.183 $\pm$ 0.005	0.210 $\pm$ 0.002
Piperacillin	0.568 $\pm$ 0.021	0.688 $\pm$ 0.111	0.223 $\pm$ 0.003	0.238 $\pm$ 0.019
Rifampicin	0.502 $\pm$ 0.008	0.509 $\pm$ 0.014	0.215 $\pm$ 0.003	0.209 $\pm$ 0.007
Tetracycline	0.538 $\pm$ 0.010	0.508 $\pm$ 0.002	0.219 $\pm$ 0.007	0.203 $\pm$ 0.001
Tigecycline	0.510 $\pm$ 0.035	0.566 $\pm$ 0.022	0.206 $\pm$ 0.014	0.225 $\pm$ 0.010
Tobramycin	0.583 $\pm$ 0.015	0.549 $\pm$ 0.009	0.241 $\pm$ 0.006	0.230 $\pm$ 0.004
Triclosan	0.596 $\pm$ 0.015	0.569 $\pm$ 0.017	0.238 $\pm$ 0.005	0.227 $\pm$ 0.008
NSC145612	0.511 $\pm$ 0.007	0.517 $\pm$ 0.009	0.218 $\pm$ 0.002	0.210 $\pm$ 0.004
Untreated	0.453 $\pm$ 0.022	0.500 $\pm$ 0.030	0.193 $\pm$ 0.008	0.213 $\pm$ 0.009
Ciprofloxacin*	0.444 $\pm$ 0.006	0.497 $\pm$ 0.013	0.183 $\pm$ 0.001	0.208 $\pm$ 0.005
Untreated*	0.369 $\pm$ 0.004	0.446 $\pm$ 0.009	0.164 $\pm$ 0.002	0.194 $\pm$ 0.004



Antibiotics	Membrane SytoxG intensity	DNA SytoxG intensity	Membrane DAPI intensity	DNA DAPI intensity
Amikacin	578.357 ± 155.218	699.618 ± 194.658	3250.040 ± 300.305	3917.849 ± 342.691
Azithromycin	32.091 ± 9.186	123.453 ± 35.671	390.001 ± 75.489	571.737 ± 56.254
Chloramphenicol	100.954 ± 33.485	126.052 ± 30.781	1387.209 ± 163.185	1324.258 ± 148.374
Colistin	20.746 ± 8.193	12.181 ± 0.581	232.524 ± 78.429	134.082 ± 40.494
Gentamicin	1689.281 ± 207.195	1915.971 ± 213.632	3794.358 ± 198.253	4094.632 ± 212.617
Kanamycin	194.450 ± 42.911	243.196 ± 53.812	2597.964 ± 169.715	3099.838 ± 219.835
Meropenem	17.774 ± 3.458	18.377 ± 3.675	253.114 ± 18.843	259.744 ± 19.356
Minocycline	80.247 ± 19.330	122.181 ± 44.699	1017.185 ± 127.827	870.702 ± 108.501
Piperacillin	10.674 ± 1.163	10.896 ± 1.167	83.445 ± 21.820	86.192 ± 22.429
Rifampicin	16.831 ± 4.743	10.566 ± 3.626	346.194 ± 23.619	256.341 ± 31.280
Tetracycline	35.513 ± 15.973	69.890 ± 43.822	967.186 ± 222.483	711.432 ± 140.700
Tigecycline	28.659 ± 7.483	88.736 ± 31.431	746.679 ± 234.490	667.615 ± 103.950
Tobramycin	324.585 ± 36.373	346.825 ± 42.551	2977.628 ± 107.192	3222.385 ± 103.431
Triclosan	44.713 ± 17.272	47.485 ± 17.983	209.222 ± 49.015	220.716 ± 50.794
NSC145612	9.749 ± 1.729	10.246 ± 2.630	84.173 ± 16.059	70.588 ± 7.783
Untreated	8.678 ± 1.711	9.412 ± 1.992	153.049 ± 28.475	125.433 ± 5.365
Ciprofloxacin*	17.132 ± 3.196	66.688 ± 18.549	340.012 ± 47.552	516.237 ± 69.061
Untreated*	12.988 ± 0.496	17.188 ± 0.913	340.799 ± 52.214	338.023 ± 68.708

Antibiotics	Membrane SytoxG standard deviation	DNA SytoxG standard deviation	Membrane DAPI standard deviation	DNA DAPI standard deviation
Amikacin	1990.149 ± 355.667	2441.234 ± 442.522	1382.508 ± 135.633	1339.156 ± 140.078
Azithromycin	109.855 ± 40.788	521.860 ± 164.923	465.287 ± 139.319	521.570 ± 77.253
Chloramphenicol	245.061 ± 60.031	474.061 ± 49.955	1353.291 ± 150.560	844.798 ± 62.395
Colistin	38.040 ± 28.854	14.734 ± 0.516	190.336 ± 59.128	78.099 ± 12.726
Gentamicin	3614.032 ± 301.627	4113.161 ± 300.704	1490.355 ± 53.410	1414.429 ± 48.963
Kanamycin	680.368 ± 130.363	864.762 ± 161.275	1334.838 ± 99.868	1369.386 ± 113.270
Meropenem	64.301 ± 11.185	66.984 ± 11.888	94.291 ± 7.856	93.357 ± 7.981
Minocycline	190.837 ± 72.488	495.425 ± 170.070	1193.115 ± 233.061	752.888 ± 103.151
Piperacillin	5.510 ± 4.810	5.387 ± 4.737	14.054 ± 6.972	12.779 ± 6.177
Rifampicin	70.773 ± 32.626	45.649 ± 29.520	458.986 ± 82.971	191.886 ± 13.439
Tetracycline	102.047 ± 61.103	230.284 ± 147.186	1438.949 ± 368.551	641.878 ± 37.431
Tigecycline	27.001 ± 7.767	262.911 ± 99.135	526.550 ± 282.648	440.378 ± 92.880
Tobramycin	1162.413 ± 163.062	1238.059 ± 175.158	1511.326 ± 79.586	1454.983 ± 80.529
Triclosan	200.386 ± 62.739	219.701 ± 66.931	146.329 ± 24.231	160.333 ± 25.120
NSC145612	13.138 ± 4.335	19.906 ± 10.102	185.832 ± 63.354	137.589 ± 11.611
Untreated	22.385 ± 11.013	23.918 ± 10.906	72.346 ± 27.259	57.232 ± 17.737
Ciprofloxacin*	40.044 ± 15.254	296.997 ± 50.929	332.964 ± 33.460	292.093 ± 38.656
Untreated*	35.950 ± 5.228	52.768 ± 4.094	202.647 ± 21.890	164.566 ± 33.649

Antibiotics	Membrane solubility, $\mu\text{m}$	DNA solubility, $\mu\text{m}$	Parent:Child	Decondensation
Amikacin	0.045 $\pm$ 0.000	0.045 $\pm$ 0.000	1.154 $\pm$ 0.126	0.904 $\pm$ 0.082
Azithromycin	0.041 $\pm$ 0.001	0.043 $\pm$ 0.000	1.165 $\pm$ 0.158	1.141 $\pm$ 0.127
Chloramphenicol	0.042 $\pm$ 0.000	0.044 $\pm$ 0.000	0.705 $\pm$ 0.273	1.209 $\pm$ 0.174
Colistin	0.044 $\pm$ 0.000	0.039 $\pm$ 0.001	0.420 $\pm$ 0.076	1.057 $\pm$ 0.115
Gentamicin	0.044 $\pm$ 0.000	0.044 $\pm$ 0.000	0.314 $\pm$ 0.065	1.154 $\pm$ 0.149
Kanamycin	0.044 $\pm$ 0.000	0.044 $\pm$ 0.000	1.036 $\pm$ 0.107	1.128 $\pm$ 0.080
Meropenem	0.045 $\pm$ 0.000	0.044 $\pm$ 0.000	1.111 $\pm$ 0.126	0.903 $\pm$ 0.213
Minocycline	0.041 $\pm$ 0.000	0.043 $\pm$ 0.000	0.892 $\pm$ 0.101	0.980 $\pm$ 0.185
Piperacillin	0.025 $\pm$ 0.001	0.025 $\pm$ 0.001	0.977 $\pm$ 0.167	1.103 $\pm$ 0.175
Rifampicin	0.043 $\pm$ 0.000	0.042 $\pm$ 0.000	0.856 $\pm$ 0.060	0.991 $\pm$ 0.076
Tetracycline	0.042 $\pm$ 0.000	0.042 $\pm$ 0.000	0.655 $\pm$ 0.073	0.987 $\pm$ 0.006
Tigecycline	0.041 $\pm$ 0.001	0.043 $\pm$ 0.000	0.783 $\pm$ 0.294	0.991 $\pm$ 0.043
Tobramycin	0.044 $\pm$ 0.000	0.044 $\pm$ 0.000	0.814 $\pm$ 0.080	0.931 $\pm$ 0.019
Triclosan	0.043 $\pm$ 0.000	0.042 $\pm$ 0.000	0.975 $\pm$ 0.187	1.212 $\pm$ 0.313
NSC145612	0.043 $\pm$ 0.000	0.041 $\pm$ 0.000	0.789 $\pm$ 0.050	0.949 $\pm$ 0.028
Untreated	0.042 $\pm$ 0.001	0.043 $\pm$ 0.000	0.957 $\pm$ 0.169	0.913 $\pm$ 0.129
Ciprofloxacin*	0.039 $\pm$ 0.000	0.042 $\pm$ 0.000	0.770 $\pm$ 0.076	0.969 $\pm$ 0.031
Untreated*	0.042 $\pm$ 0.000	0.043 $\pm$ 0.000	0.969 $\pm$ 0.031	1.250 $\pm$ 0.066

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91 \*Data obtained from *A. baumannii* ATCC 17978

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94 **Table S3.** MIC of NSC145612 and rifampicin in *E. coli* strains with and without *rpoB*  
 95 mutations.

Strains	<i>rpoB</i> mutations	MIC	
		NSC145612 ( $\mu\text{M}$ )	Rifampicin ( $\mu\text{M}$ )
<i>E. coli</i>	-	30	9
<i>E. coli</i> LB141	<i>rpoB</i> (H526Y)	>200	>200
<i>E. coli</i> LB142	<i>rpoB</i> (S509_L511del)	>200	>200
<i>E. coli</i> LB143	<i>rpoB</i> (V146F)	>200	>200
<i>E. coli</i> LB144	<i>rpoB</i> (H526Y)	>200	>200

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