

1 **Supporting Information Legend**

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3 **Supplemental Table 1.** Minimum Inhibitory Concentrations (MIC<sub>90</sub>) for the antibiotics and iron  
4 chelators used in this study. The concentrations reported were the concentrations used in the  
5 assays of this report. All concentrations are in µg/ml except DIP that is in µM. Antibiotics used:  
6 ampicillin (AMP), cefotaxime (CTX), chloramphenicol (CHL), ciprofloxacin (CIP), daptomycin  
7 (DAP), gentamicin (GEN), levofloxacin (LVX), meropenem (MEM) methicillin (MET),  
8 spectinomycin (SPT), trimethoprim (TMP), vancomycin (VAN), deferiprone (DFP),  
9 deferoxamine (DFX), and 2,2'-dipyridyl (DIP). Data represent mean and standard deviation of at  
10 least six independent replicates.

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|     | <i>E. coli</i> ExPEC CP9 | <i>S. aureus</i> TCH1516 |
|-----|--------------------------|--------------------------|
| AMP | 5                        | 2000                     |
| CTX | 0.125                    | N/A                      |
| CHL | 1000                     | 10                       |
| CIP | 0.0625                   | N/A                      |
| DAP | N/A                      | 50                       |
| GEN | 12.5                     | N/A                      |
| LVX | 0.1                      | 0.5                      |
| MET | 2500                     | 40                       |
| MEM | 0.04                     | N/A                      |
| SPT | 50                       | 250                      |
| TMP | 1                        | N/A                      |
| VAN | 1000                     | 10                       |
| DFP | 300                      | >1200                    |
| DFX | >4000                    | >2400                    |
| DIP | 500                      | >2500                    |

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28 **Supplemental Table 2.** Classification and summary of the ferricidal effect of antibiotics tested  
 29 in the study. “Y”=yes, the terminal OD<sub>600</sub> (OD<sub>600-18h</sub> – OD<sub>600-0h</sub>) for the antibiotic+chelator  
 30 treatment was lower than each compound separated; “N”= no growth; “N/A”= not applicable.

| Name | Class           | Mechanism                          | Growth<br>Inhibition<br>(+DFP)<br><i>E. coli</i> | Growth<br>Inhibition<br>(+DFX)<br><i>E. coli</i> | Growth<br>Inhibition<br>(+DIP)<br><i>E. coli</i> | Growth<br>Inhibition<br>(+DFP)<br><i>S. aureus</i> |
|------|-----------------|------------------------------------|--|--|--|--|
| AMP  | β-lactam        | inhibits cell wall<br>synthesis    | Y  | N  | Y  | Y  |
| CTX  | β-lactam        | inhibits cell wall<br>synthesis    | Y  | N  | Y  | N/A  |
| CHL  | chloramphenicol | inhibits 50S<br>ribosomal subunit  | Y  | Y  | Y  | N  |
| CIP  | fluoroquinolone | inhibits DNA gyrase                | N  | N  | N  | N/A  |
| DAP  | lipopeptide     | disrupts membrane<br>integrity     | N/A  | N/A  | N/A  | N  |
| GEN  | aminoglycoside  | inhibits 30S<br>ribosomal subunit  | N  | N  | N  | N/A  |
| LVX  | fluoroquinolone | inhibits type II<br>topoisomerases | N  | N  | N  | Y  |
| MET  | β-lactam        | inhibits cell wall<br>synthesis    | Y  | N  | N  | N/A  |
| MEM  | β-lactam        | inhibits cell wall<br>synthesis    | N  | N  | N  | N  |

|     |   |  |   |   |   |     |
|-----|---|--|---|---|---|-----|
| SPT | aminocyclitol                           | inhibits 30S<br>ribosomal subunit      | N | N | N | N   |
| TMP | dihydrofolate<br>reductase<br>inhibitor | inhibits DNA<br>synthesis              | N | N | N | N/A |
| VAN | glycopeptide                            | inhibits<br>peptidoglycan<br>synthesis | Y | N | Y | N   |

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44 **Supplementary Table 3.** The known or published properties of three iron chelators (deferiprone,  
 45 2,2'-dipyridyl, and deferoxamine). Listed are the antibiotics that demonstrate enhanced  
 46 antibacterial activity when combined with the chelator, their ability to cross a membrane bilayer,  
 47 molecular weight, and published affinity for iron.

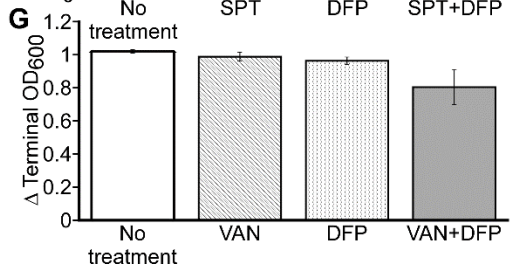
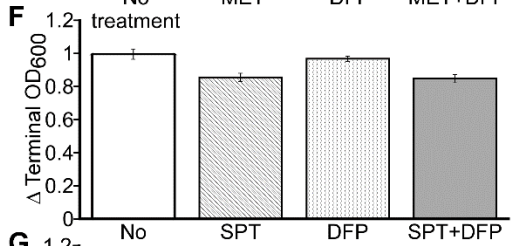
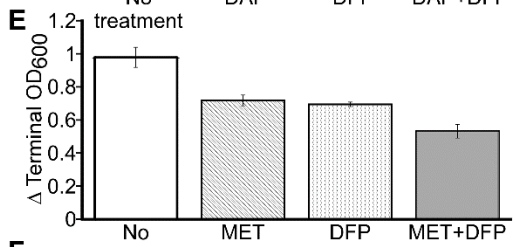
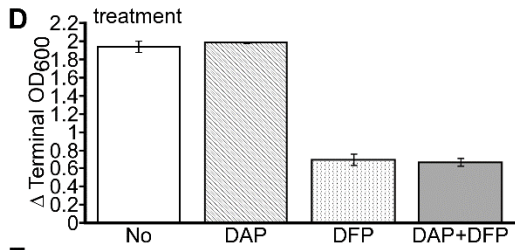
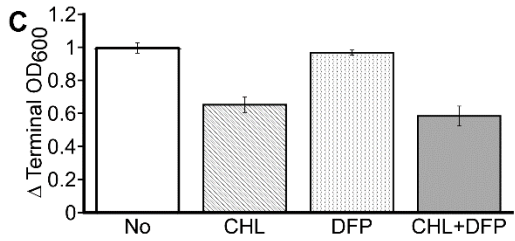
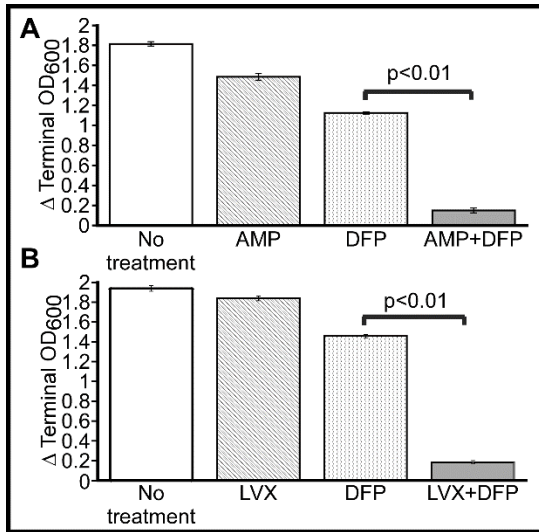
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| Name           | Abbreviation | Antibacterial activity with:    | Ability to enter cells | Size (g/mol) | Affinity for iron  | Reference |
|----------------|--------------|---------------------------------|------------------------|--------------|--|-----------|
| Deferiprone    | DFP          | AMP<br>CHL<br>CTX<br>MET<br>VAN | high                   | 139.15       | $1 \times 10^{19} \text{ M}^{-1}$<br>(Fe <sup>3+</sup> )   | (1, 2)    |
| 2,2'-Dipyridyl | DIP          | AMP<br>CHL<br>CTX<br>VAN        | high                   | 156.18       | $1 \times 10^{16} \text{ M}^{-1}$<br>(Fe <sup>3+</sup> )<br>$1 \times 10^{17} \text{ M}^{-1}$<br>(Fe <sup>2+</sup> ) | (3, 4)    |
| Deferoxamine   | DFX          | CHL                             | low                    | 560.68       | $1 \times 10^{30} \text{ M}^{-1}$<br>(Fe <sup>3+</sup> )   | (1, 5)    |

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53 **Supplemental Figure 1.** Iron chelators and antibiotics restrict the growth of *S. aureus*. *S. aureus*  
54 TCH1516 was cultured in BHI in the presence or absence of antibiotic, DFP (900 µg/ml), or both  
55 for 18 hrs. and the terminal OD<sub>600</sub> (OD<sub>600-18h</sub> – OD<sub>600-0h</sub>) was measured as described in the  
56 Experimental Procedures. (A) AMP (500 µg/ml), (B) LVX (0.1 µg/ml), (C) CHL (2.5 µg/ml), (D)  
57 DAP (10 µg/ml), (E) MET (10 µg/ml), (F) SPT (50 µg/ml), (G) VAN (1 µg/ml). Data represent  
58 the mean and standard deviation of at least six separate replicates. The *p* value was determined  
59 by a Student's t-test. The black box indicates the antibiotics that showed enhanced bacterial  
60 growth inhibition when combined with DFP.

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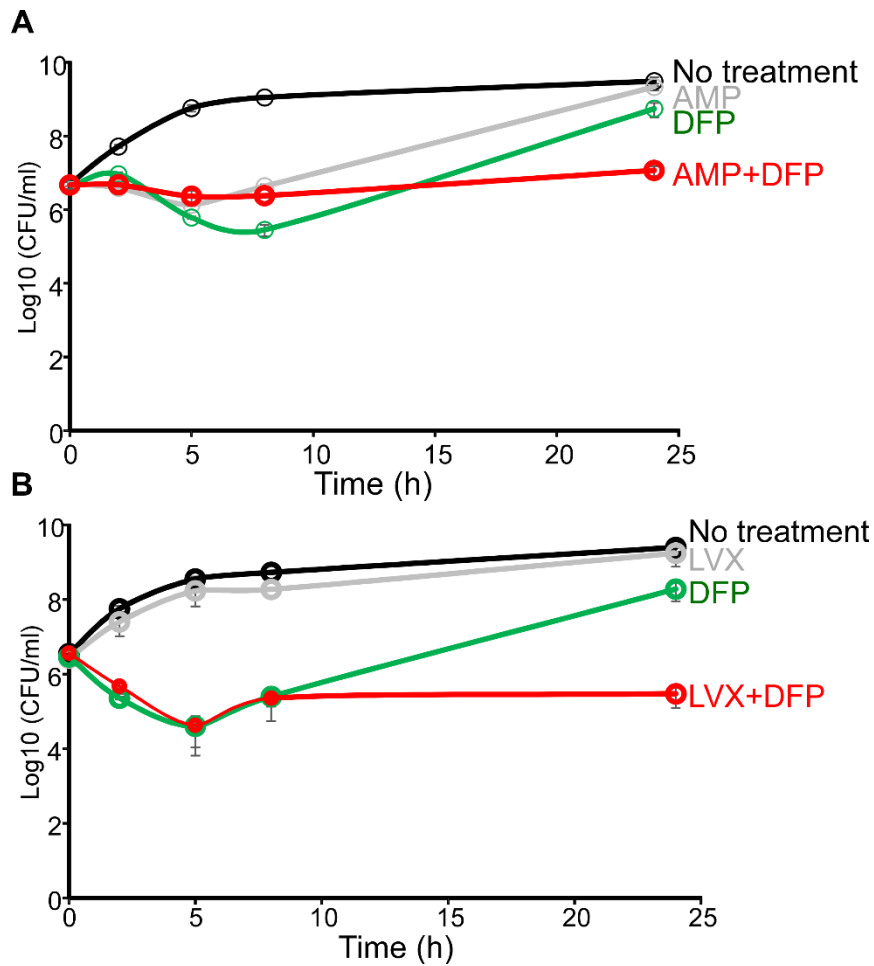
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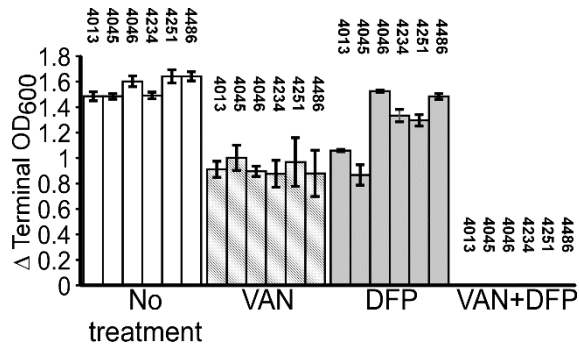


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75 **Supplemental Figure 2.** Time-kill assays for the antibiotics and DFP that demonstrate an  
 76 enhanced efficacy towards *S. aureus*. Bacteria were cultured in 5 mL BHI overnight at 37 °C with  
 77 vigorous shaking and then subcultured into 5 mL of Cation-Adjusted Mueller-Hinton Broth  
 78 (CAMHB) at a starting OD600 = 0.01, supplemented with antibiotics: (A) AMP (500 µg/ml), (B)  
 79 LVX (0.1 µg/ml), DFP (900 µg/ml), antibiotic with DFP, or not supplemented at all. CFUs of  
 80 each culture were determined by 10 fold serial dilutions and drip-plating of 20 µL of each diluent  
 81 at the times indicated. Data represent the mean and standard deviation of three independent  
 82 experiments.

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85 **Supplemental Figure 3.** The effect of vancomycin and DFP on the growth of clinical *E. coli*

86 isolates. All growth assays were performed in 96 well plates in 200 μl of BHI at a starting

87 OD<sub>600</sub>=0.01. Antibiotics and chelators were added to the well when appropriate at the following

88 concentrations: ELZ4013: 100 μg/ml VAN, 125 μg/ml DFP; ELZ4045: 250 μg/ml VAN, 300

89 μg/ml DFP; ELZ4046: 500 μg/ml VAN, 300 μg/ml DFP; ELZ4234: 400 μg/ml VAN, 300 μg/ml

90 DFP; ELZ4251: 2 mg/ml VAN, 125 μg/ml DFP; and ELZ4486: 500 μg/ml VAN, 300 μg/ml

91 DFP. Bacterial growth was quantified by determining the difference in the optical density

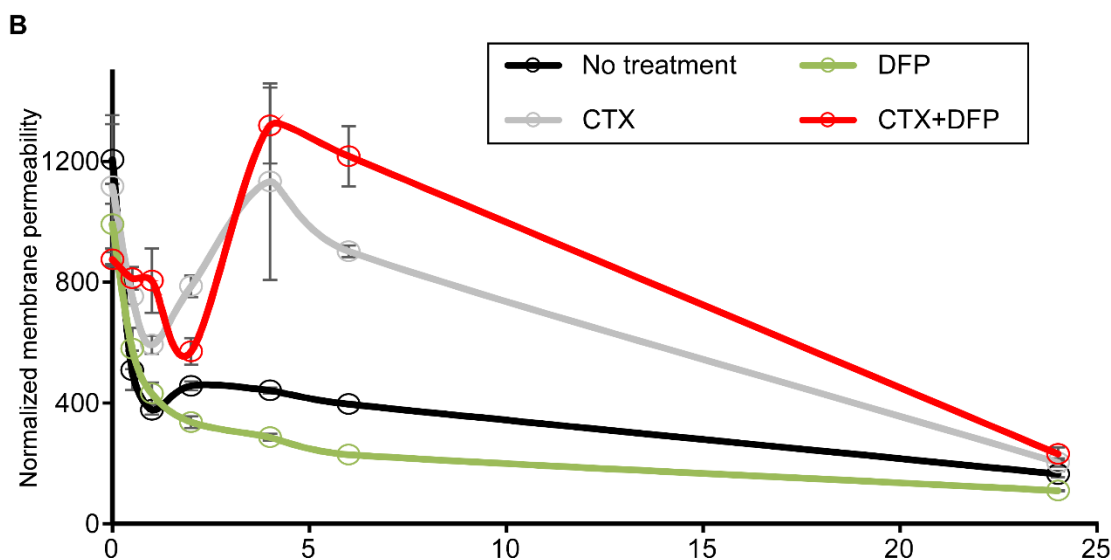
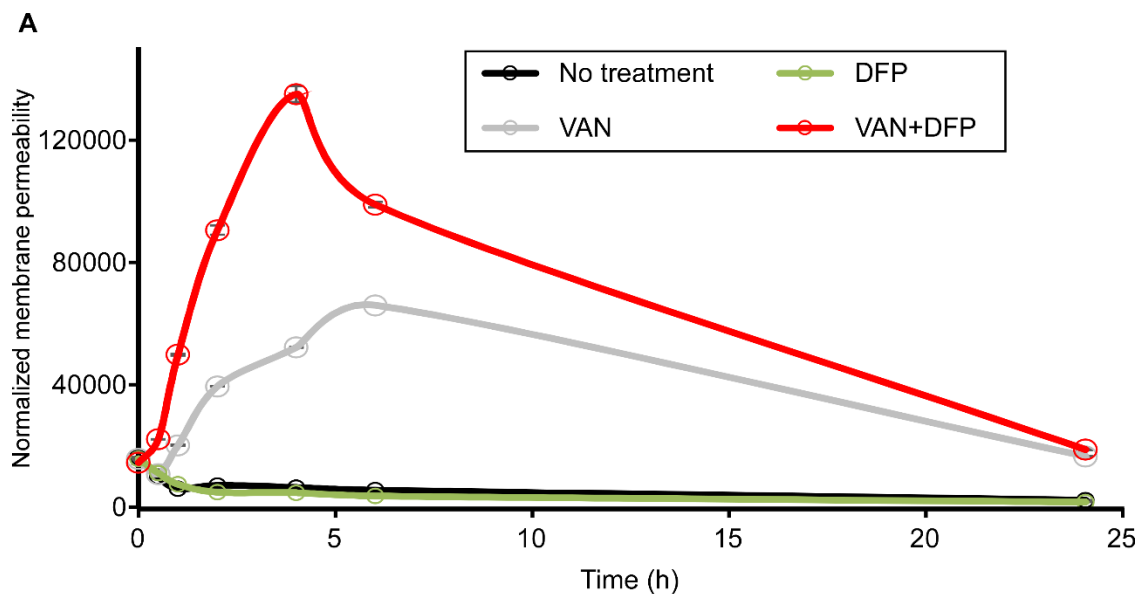
92 between the start and end of the experiment, represented as OD<sub>600-18h</sub> – OD<sub>600-0h</sub>. Data represent

93 the mean and standard deviation of three independent experiments.

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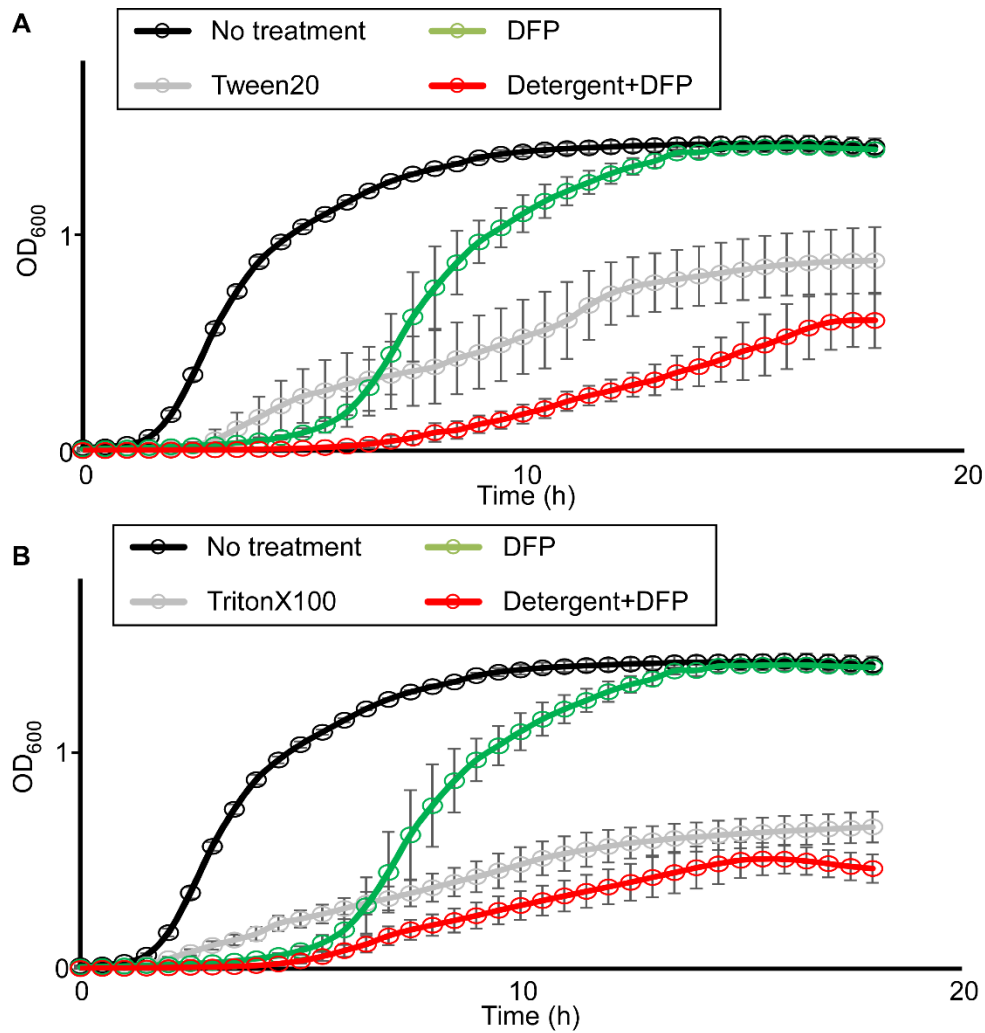
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**Supplemental Figure 4.** The effect of antibiotic and DFP on membrane permeability. *E. coli* CP9 was cultured in the presence and absence of antibiotics (VAN-250  $\mu\text{g/ml}$ , CTX-62.5 ng/ml), 150  $\mu\text{g/ml}$  DFP, or both. Fluorescence, which positively correlated with membrane permeability, were measured at the indicated times. Data were expressed as fluorescence per cell and represent the mean and standard deviation of three independent experiments.



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**Supplemental Figure 5.** The effect of detergents and DFP on the growth of ExPEC. *E. coli*

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strain CP9 was cultured in the presence and absence of detergents (A - Tween20 and B - Triton-

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100, both at 20%), DFP (150 µg/ml), or both detergent and DFP and growth (OD<sub>600</sub>) were

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measured for 18 hrs. The data represent the mean and standard deviation of three independent

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experiments.

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