a Fluoroquinolones (FQ)

Ciprofloxacin Levofloxacin Moxifloxacin

b Aminoglycosides (AG)

Gentamicin Tobramycin Amikacin

squared projected disatance (SPD)

E. coli

K. pneumoniae

c Beta-Lactams (BL)

Ampicillin Cefazolin Ceftriaxone Aztreonam Piperacillin/Tazobactam Ertapenem Meropenem

squared projected disatance (SPD)

E. coli

K. pneumoniae

CLSI classification

1

2 **FIG S1. One-dimensional projection of NanoString data distinguishes susceptible**

3 **from resistant isolates.** Panels show one-dimensional projections (squared projected

4 distance, SPD (1, 2)) of NanoString heatmap data from Fig. 1 for clinical isolates of *E.*

5 *coli* (top panels) and *K. pneumoniae* (bottom panels) treated across (a) fluoroquinolones,

6 (b) aminoglycosides, and (c) beta-lactams, binned by CLSI classifications (S, susceptible;

7 I, intermediate; R, resistant). By definition, an SPD of 0 indicates a transcriptional

8 response to antibiotic equivalent to that of an average susceptible strain, while an SPD

9 of 1 indicates a response equivalent to that of an average resistant strain. Data are

10 summarized as box-and-whisker plots in the style of Tukey (3), where boxes extend from

11 the 25th to 75th percentile for each category, with a line at the median, and whiskers extend

12 from the minimum to the maximum.

a *E. coli +* ertapenem

b *K. pneumoniae +* ertapenem

13
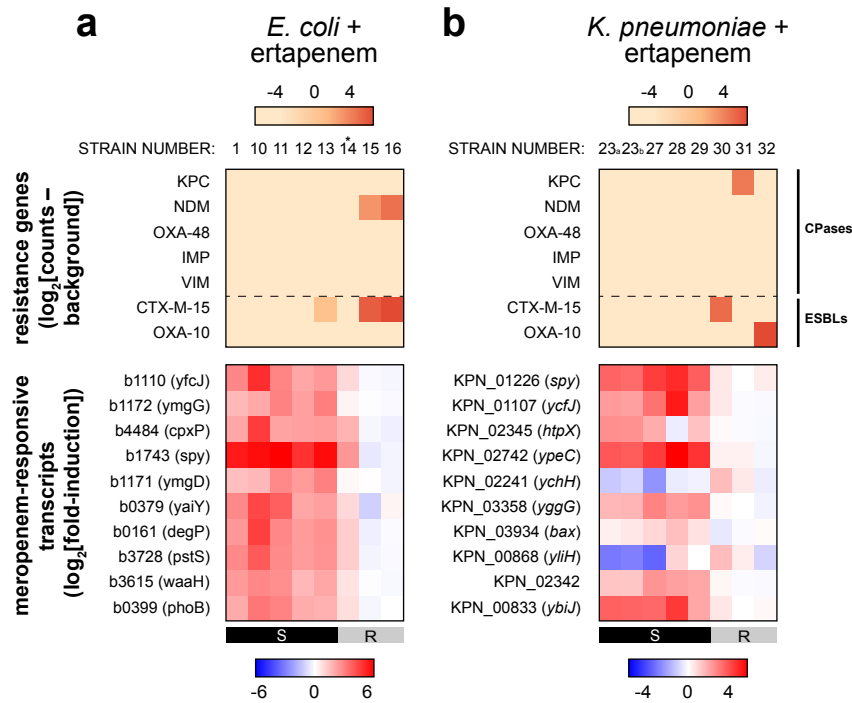
FIG S2. GoPhAST-R detects carbapenemase (CPase) and extended-spectrum beta-

lactamase (ESBL) gene content, augmenting phenotypic AST. Top panels show

GoPhAST-R detection of select CPase and ESBL transcript content in (**a**) *E. coli* and (**b**)

K. pneumoniae strains selected for BL treatment. Heatmap intensity reflects normalized,

background-subtracted, log-transformed NanoString data from probes for the indicated

gene families, as described (1). Color scales indicate range of $\log_2$[counts – background]

for these genes in the respective heatmap(s). Bottom panels show the GoPhAST-R

phenotypic AST heatmaps for ertapenem treatment from Fig. 1c. *Note strain 14 displays

phenotypic ertapenem resistance without detectable CPase or ESBL from our panel.

23

24   **DATASET S1. Clinical isolates included in this study.** Table shows the strains used

25   in this study with corresponding isolate source, relevant MICs, relevant resistance

26   mechanisms found by WGS analysis with ResFinder, and NCBI BioSample number.

27   Genotypic resistance determinants are color-coded by three general mechanisms of

28   resistance: permeability alterations (blue), target site modifications (orange), and drug

29   modifications (purple).

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31   References
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