The carbapenem inoculum effect provides insight into the molecular mechanisms underlying carbapenem resistance in Enterobacterales

Alexis Jaramillo Cartagena[1†], Kyra L. Taylor[1†], Leslie C. Lopez[1†], Jennifer Su[1,2], Joshua T. Smith[1], Abigail L. Manson[1], Jonathan D. Chen[1,3], Virginia M. Pierce[4,5], Ashlee M. Earl[1], Roby P. Bhattacharyya[1,6,*]

[1]Infectious Disease and Microbiome Program, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA
[2]Biological and Biomedical Sciences Graduate Program, Harvard Medical School, Boston, MA 02115, USA
[3]Harvard University, Cambridge MA 02138
[4]Microbiology Laboratory, Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA
[5]Department of Pathology and Clinical Laboratories, University of Michigan, Ann Arbor, MI 48109, USA
[6]Infectious Diseases Division, Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
[†]These authors contributed equally to this work.

*Corresponding Author

Email: rbhatt@broadinstitute.org

Author Contributions:
Designed research: AJC, KLT, LCL, JS, RPB
Performed research: AJC, KLT, LCL, JS, JDC, RPB
Contributed new reagents/analytic tools: AJC, KLT, AME, RPB
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Abstract

Carbapenem-resistant Enterobacterales (CRE) are important pathogens that can develop resistance via multiple molecular mechanisms, including hydrolysis or reduced antibiotic influx. Identifying these mechanisms can improve pathogen surveillance, infection control, and patient care. We investigated, both phenomenologically and mechanistically, how resistance mechanisms influence the carbapenem inoculum effect (IE), a phenomenon where inoculum size affects antimicrobial susceptibility testing (AST). We demonstrated that any of seven different carbapenemases were sufficient to impart a meropenem IE when transformed into a laboratory strain of *Escherichia coli*. Across 106 clinical CRE isolates spanning 6 genera and 12 species, the carbapenem IE strictly depended on resistance mechanism: all 36 carbapenemase-producing CRE (CP-CRE) exhibited a clear IE, whereas 43 porin-deficient CRE displayed none. 27 isolates with both carbapenemase production and porin deficiency exhibited high-level resistance at all inocula, and still displayed an IE, albeit smaller in magnitude than CP-CRE with intact porins. Mechanistically, we found that clinical CP-CRE release carbapenemase activity into culture supernatant to protect other cells. Further, this released activity markedly increased upon exposure to lethal antibiotic doses, functionally consistent with altruism. Concerningly, 50% and 24% of CP-CRE isolates changed susceptibility classification to meropenem and ertapenem, respectively, across the allowable inoculum range in clinical testing guidelines. The meropenem IE, and the ratio of ertapenem to meropenem minimal inhibitory concentration (MIC) at standard inoculum, reliably identified CP-CRE. Understanding how resistance mechanisms affect AST could improve diagnosis and guide therapies for CRE infections.

58 **Importance**

59

60 Infections caused by carbapenem-resistant Enterobacterales (CRE) pose significant threats to
61 patients and public health worldwide. Carbapenem resistance can occur through several
62 molecular mechanisms, including enzymatic hydrolysis by carbapenemases and reduced influx
63 via porin mutations. Identifying carbapenemase-producing isolates could enable tailored
64 antibiotic selection to improve patient outcomes, and infection control measures to prevent
65 further carbapenemase transmission. In a large collection of CRE isolates, we found that only
66 carbapenemase-producing CRE exhibit an inoculum effect, in which their measured resistance
67 varies markedly with cell density, which risks misdiagnosis. Further, this inoculum effect occurs
68 under conditions where bacteria release carbapenemases to the community upon exposure to
69 antibiotic that results in cell death, functionally consistent with altruism. Measuring this inoculum
70 effect, or integrating other data from routine antimicrobial susceptibility testing, enhances
71 carbapenem resistance detection, paving the way for more effective strategies to combat this
72 growing public health crisis.

73 **Main Text**

74

75 **Introduction**

76

77 Carbapenems are a crucial class of β-lactam antibiotics with a broad spectrum of activity (1) due
78 to their ability to withstand hydrolysis by many β-lactamases and their inhibition of cell wall
79 synthesis across many bacterial species (2). The emergence of carbapenem-resistant
80 Enterobacterales (CRE) poses a significant public health risk, with the US Centers for Disease
81 Control and Prevention (CDC) designating them as an "urgent threat" due to their extensive
82 drug resistance, high mortality rates, and treatment challenges (3, 4). The rapid global
83 emergence of CRE has created an urgent clinical and epidemiological need to better
84 understand, detect, and limit the spread of these pathogens (5).

85 CRE employ two major molecular mechanisms of carbapenem resistance: 1) expression
86 of carbapenemases, which efficiently hydrolyze carbapenems, or 2) disruption of porins, which
87 reduces influx of carbapenems into the periplasm where they act (6). This reduced periplasmic
88 influx can potentiate β-lactamases with weak carbapenemase activity such as AmpC, CTX-M,
89 SHV, TEM, or OXA-10, which together cause resistance (1, 7). Efflux pumps can also contribute
90 to carbapenem resistance (8), though their role has been better characterized in non-
91 Enterobacterales species like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (9, 10).
92 Carbapenemase-producing CRE (CP-CRE) are of particular epidemiological concern due to
93 their propensity to cause outbreaks (4, 11) and the risk of horizontal transfer of carbapenemase
94 genes (12-14). Porin deficient CRE (PD-CRE) often exhibit impaired fitness (15) compared to
95 CP-CRE because porins play important roles in nutrient import (16), osmotic homeostasis (15,
96 17), and membrane lipid asymmetry (18). Determining resistance mechanisms could lead to
97 better infection control measures, such as patient isolation to prevent CP-CRE spread in
98 hospitals (4, 13, 19). Many laboratories in high-resource settings now test for resistance

99 mechanisms in CRE (20-22), but this requires additional time, equipment, expertise, and
100 expenses. Capitalizing on existing antimicrobial susceptibility testing (AST) workflows to predict
101 CRE resistance mechanisms and enhance efficiency of molecular testing deployment would
102 represent a clinical advance.

103 Standardized AST methods and interpretive criteria are crucial to determine
104 antimicrobial susceptibility of pathogens. Organizations like the Clinical and Laboratory
105 Standards Institute (CLSI) classify pathogen susceptibility based on minimum inhibitory
106 concentration (MIC) breakpoints (23-25) that are established and periodically revised (26) based
107 on microbiological, pharmacokinetic-pharmacodynamic, and clinical data. While these
108 breakpoints are intended to predict clinical response to therapy, not resistance mechanism,
109 carbapenem MICs have been repurposed to prioritize isolates for molecular carbapenemase
110 testing (25, 27). In the United States, only isolates that test phenotypically carbapenem resistant
111 are typically tested for carbapenemases (24). Yet despite recently updated breakpoints (26),
112 some carbapenemase producing isolates can still be misclassified as susceptible to
113 carbapenems (28).

114 The inoculum effect (IE) is a long-recognized phenomenon whereby the measured MIC
115 varies depending on the precise bacterial inoculum used in AST assays, which can lead to
116 susceptibility misclassification and treatment failures (29-31). Measured MICs can vary
117 markedly because of the IE, with high inocula withstanding antibiotic concentrations hundreds of
118 fold higher than low inocula, consistent with the majority of resistance emerging from the
119 community, rather than individual bacteria. Multiple factors are proposed to contribute to the IE,
120 including metabolic state of the bacteria (32), growth productivity (33), antibiotic-target ratio (34),
121 heteroresistance frequency (35), target proteolysis (36), and production of enzymes that
122 inactivate antibiotics (37-41). The IE is frequently observed for some antibiotic classes including
123 β-lactams (31, 42) but not commonly for others such as aminoglycosides. In *Staphylococcus*
124 *aureus*, where the cefazolin IE has been thoroughly studied, β-lactamase carriage appears to

125 be necessary but not sufficient to predict an IE (43-45). While the importance of the IE in clinical
126 infections remains uncertain (46, 47), it has important implications for diagnostic accuracy. To
127 minimize the impact of the IE, CLSI recommends a standard inoculum of 5×10^5 colony forming
128 units (CFU)/mL, with an acceptable range of 2×10^5 to 8×10^5 CFU/mL, for broth microdilution,
129 the gold standard AST method (24). Even within this range, however, carbapenem susceptibility
130 classification of Enterobacterales isolates may change due to the IE, underscoring the need for
131 continued efforts to optimize AST methods (48). Thus, clarity on the mechanistic basis for the IE
132 in the Enterobacterales has diagnostic and potentially therapeutic implications.

133 Here, we sought to systematically investigate how different genotypes conferring
134 carbapenem resistance influence the IE and how understanding these relationships might be
135 leveraged to improve clinical AST. We found that expression of distinct carbapenemases was
136 sufficient to cause a marked meropenem IE in a laboratory strain of *Escherichia coli*. Further, in
137 a collection of 106 clinical CRE isolates across 12 species of Enterobacterales (12, 14, 49), we
138 observed that the carbapenem IE was fully predicted by resistance mechanism. Specifically, all
139 CP-CRE exhibited a strong carbapenem IE, whereas all PD-CRE exhibited constant
140 carbapenem MICs across a wide range of inocula. Isolates with both a carbapenemase and
141 porin deficiency generally exhibited an IE and higher MICs than strains with either mechanism
142 alone. Release of carbapenemase activity from clinical CP-CRE into the supernatant to confer
143 transferrable protection to other cells was markedly enhanced upon exposure to lethal antibiotic
144 doses, providing a plausible mechanistic basis for community protection, functionally consistent
145 with altruism from antibiotic-mediated cell death. The carbapenem MICs of CP-CRE varied
146 considerably, with a large proportion changing AST classification across the CLSI-acceptable
147 inoculum range. Collectively, our results demonstrated that the IE distinguished CRE by
148 resistance mechanism, better than either meropenem or ertapenem MIC alone. These findings
149 suggested two phenotypic measures that predict carbapenem resistance mechanisms better
150 than the MIC cutoffs currently used to prioritize molecular carbapenemase testing.

151

152 **Results**

153

154 **Carbapenemases belonging to all four Ambler classes impart an inoculum effect.**

155 Carbapenemases are commonly divided into four classes based on protein sequence and
156 enzymatic features (50, 51). To investigate if each carbapenemase class is sufficient to confer
157 an IE, we systematically cloned seven different carbapenemase genes, each with its native
158 promoter, into a low-copy pBAD33 plasmid (52): bla_{KPC-3} and bla_{SME-2} from Ambler class A;
159 bla_{IMP-4}, bla_{NDM-1}, and bla_{VIM-27} from class B; bla_{CMY-10} (53) from class C; and bla_{OXA-48} from class D
160 (Supplementary Table S1). We transformed each carbapenemase-encoding plasmid into *E. coli*
161 K-12 MG1655, a carbapenem-susceptible laboratory strain, then conducted meropenem broth
162 microdilution MIC assays across a broad range of inocula, comprising two-fold dilutions from 1.3
163 $\times 10^7$ to 1.6×10^3 CFU/mL. As inoculum increased, all transformants showed a significant
164 increase in MIC, while the MIC of the empty vector control did not (Fig. 1*A*). Most transformants
165 had stable meropenem MICs from ~10^3 to ~10^4 CFU/mL, and all exhibited a strong linear
166 increase in MIC across the ~10^5 to ~10^7 CFU/mL inoculum range, which includes the
167 recommended inoculum range for clinical testing (Fig. 1*B*). In this strain background, NDM-1
168 (orange), SME-2 (pink), and IMP-4 (brown) imparted the highest levels of resistance, followed
169 by VIM-27 (yellow). KPC-3 (teal) conferred high-level resistance at high inocula, but within the
170 CLSI inoculum range (dashed lines in Fig. 1*B*) MICs remained within the intermediate
171 classification (>1 μg/mL and <4 μg/mL). OXA-48 (violet) and CMY-10 (green) conferred the
172 lowest meropenem MICs, which remained below 1 μg/mL until the highest inoculum tested and
173 well within the susceptible classification in the CLSI range. The extended-spectrum beta-
174 lactamase (ESBL) gene $bla_{CTX-M-15}$, which often contributes to a CRE phenotype when paired
175 with porin deficiency (54, 55), conferred only a minimal IE, similar to empty vector
176 (Supplementary Fig. S1).

177

178 **Inoculum effect of clinical CRE isolates depends on resistance mechanism.** After
179 establishing that all carbapenemases imparted a meropenem IE in *E. coli* K-12, we investigated
180 the IE in clinical isolates harboring carbapenemases, porin disruptions, or both. We analyzed
181 106 clinical CRE isolates comprising 6 genera and 12 species (Supplementary Table S2), which
182 we assigned to three groups based on their genotypes: CP-CRE, PD-CRE, and CP-CRE with
183 porin deficiency (Fig. 2 and Supplementary Fig. S2, Table S2 and Table S3). We observed that
184 all 36 CP-CRE harboring carbapenemases from one of six major families (KPC, NDM, IMP,
185 VIM, OXA-48, and SME) displayed a meropenem IE similar to the *E. coli* K-12 carbapenemase
186 transformants (Fig. 2*A* and Supplementary Fig. S2*A*). In contrast, 43 PD-CRE, many of which
187 encoded ESBLs, exhibited a strikingly different pattern of resistance, with nearly constant MICs
188 across the tested inoculum range (Fig. 2*B* and Supplementary Fig. S2*B*). The 27 isolates with
189 both carbapenemase production and porin deficiencies, which we termed "hyper-CRE",
190 exhibited an IE and higher-level resistance across all tested inocula than isolates containing
191 only one mechanism of resistance (Fig. 2*C* and Supplementary Fig. S2*C*). 5 ESBL-encoding
192 meropenem-susceptible isolates exhibited no meropenem IE, nor did 5 other randomly selected
193 meropenem-susceptible isolates (Supplementary Fig. S2*D*).

194

195 **Carbapenemase production and porin deficiency together cause a hyper-CRE phenotype.**
196 To directly test the hypothesis that the combination of carbapenemase production and porin
197 deficiency confers a hyper-CRE phenotype, we selected two clinical *Klebsiella pneumoniae*
198 isolates (BIDMC46a and BIDMC91) with disruptions in both major porins OmpK36 and
199 OmpK35, and transformed them with a pBAD33 plasmid encoding a KPC-3 (Fig. 3*A*). Both
200 transformants exhibited dramatic increases in MIC at standard inoculum (256-fold and 128-fold,
201 respectively) compared to empty vector controls and also developed an IE (6-fold from standard
202 to high inoculum). In a second experiment, we disrupted the major OmpK36 porin in a *K.*

203 *pneumoniae* isolate expressing a KPC-2 (RB582) using a CRISPR-Cas9 base editor (56).
204 Meropenem MICs at standard inoculum increased by 32-fold in the edited strain, with a small IE
205 (2-fold from standard to high inoculum, Fig. 3*B*). These experiments provide direct evidence that
206 the combination of carbapenemase production and porin deficiency leads to a hyper-CRE
207 phenotype.
208
209 **Antibiotic exposure enhances CP-CRE release of carbapenemase activity into media.** The
210 strict dependence of the IE upon resistance genotype suggests that carbapenemase production
211 may directly cause the inoculum effect. One plausible molecular mechanism is that
212 carbapenemases are shared as common goods protecting bacterial populations (57). To test
213 this hypothesis, we set up supernatant transfer experiments (Fig. 4*A*) to ask whether CP-CRE,
214 or PD-CRE as controls (each referred to as "producer strains" in this experimental setup),
215 release carbapenemase activity that can protect a fully susceptible reporter strain, *E. coli* K-12.
216 Surprisingly, supernatant from cultures of three clinical CP-CRE grown to high inoculum (1×10^7
217 CFU/mL), each from a different species and expressing a different carbapenemase, had no
218 effect on reporter strain MIC at baseline (Fig 4*B* top panel, dashed lines). However, upon
219 treatment with meropenem, supernatant from these high-inoculum cultures protected the K-12
220 reporter strain, increasing its meropenem MIC by up to >100-fold (Fig. 4*B* top panel, solid lines).
221 By contrast, supernatant from high-inoculum PD-CRE cultures had no effect on the meropenem
222 MIC of the reporter strain, with or without meropenem treatment (Fig. 4*B* bottom panel, solid vs
223 dashed lines, respectively). Notably, we treated each producer strain with meropenem at half its
224 respective standard-inoculum MIC, which for the tested CP-CRE strains was 16- to 128-fold
225 below their MIC at the high inoculum at which we treated them, but 1- to 8-fold above their low-
226 inoculum MIC (these relationships varied depending on the shape of the IE curve for each
227 strain). Despite treating so far below each producer strain's MIC at the treated (high) inoculum,

228 we nonetheless observed death or reduced growth of CP-CRE strains in the initial 2 hours of
229 treatment (Supplementary Fig. S3).
230 To test whether this phenomenon generalized to all carbapenemases, we repeated
231 these supernatant transfer experiments using our laboratory strains of *E. coli* K-12 transformed
232 with each carbapenemase as producer strains (Supplementary Fig. S4). Each transformant
233 generated transferrable carbapenemase activity after meropenem treatment (Supplementary
234 Fig. S4*B*). In this laboratory strain background, unlike in the clinical producers, the stronger
235 carbapenemases (all but OXA-48) generated detectable transferrable protection in the absence
236 of antibiotics as well (dashed lines), though in all cases the amount of protection increased
237 markedly with antibiotic treatment (solid lines).
238 This transferrable carbapenemase activity in the supernatant was released (from clinical
239 CP-CRE strains) or enhanced (from K-12 transformants) not only by meropenem treatment, but
240 also by treatment with the aminoglycoside apramycin (Supplementary Figs. S4*C* and S5), an
241 unrelated antibiotic to which all strains were susceptible. Consistent with this protective activity
242 coming from released carbapenemase, the selective carbapenemase inhibitor vaborbactam fully
243 reversed the protection from KPC- and SME-producing strains, but not from strains producing
244 the class B or D β-lactamases NDM, IMP, VIM, or OXA-48 (Fig. 4, Supplementary Figs. S4 and
245 S5, red lines), matching vaborbactam's known spectrum of activity (58).
246
247 **A significant proportion of carbapenemase-producing isolates change classification**
248 **within the CLSI inoculum range.** Although clinical microbiological laboratories initially
249 identified the isolates we studied as carbapenem-resistant, the IE caused the MIC of several
250 isolates to vary greatly within the CLSI inoculum range (Fig. 2*A*, and Supplementary Fig. S2*A*).
251 This variability prompted us to quantify which carbapenemase-producing isolates changed
252 susceptibility classification within the acceptable CLSI inoculum range of 2×10^5 CFU/mL to $8 \times$
253 10^5 CFU/mL. We tested both meropenem and ertapenem, two carbapenems in common clinical

254 use with distinct physicochemical and pharmacokinetic properties. Notably, ertapenem has a
255 lower barrier to resistance (59) since it is more affected by reduced outer membrane
256 permeability (60), and has lower clinical susceptibility breakpoints (24). 22 of 63 (35%)
257 carbapenemase-producing isolates tested with meropenem and 7 of 41 (17%) tested with
258 ertapenem changed AST classification (Fig. 5). When excluding hyper-CRE and considering
259 only isolates with intact porins, 18 of 36 (50%) CP-CRE isolates changed AST classification for
260 meropenem and 7 of 29 (24%) for ertapenem within the CLSI inoculum range. Concerningly,
261 three isolates changed from meropenem susceptible to resistant within the CLSI range (Fig. 4,
262 dotted lines), including one *E. coli* isolate (BIDMC43b) and two *Serratia marcescens* isolates
263 (BWH56 and BWH57), each of which encodes a KPC-3. Four isolates tested fully susceptible to
264 meropenem throughout the CLSI inoculum range (red circle), including two *Citrobacter freundii*
265 harboring KPC-3 (MGH281 and MGH283), and one *E. coli* (BAA2523) and one *K. pneumoniae*
266 (BAA2524) each harboring an OXA-48. In all, 15 of 63 (24%) carbapenemase producers (CP-
267 CRE or hyper-CRE) tested meropenem susceptible, and 2 of 41 (4.9%) tested ertapenem
268 susceptible, at some point in the CLSI inoculum range; this represented 15 of 36 (42%) and 2 of
269 29 (6.9%) CP-CRE, respectively.
270
271 **The inoculum effect accurately identifies carbapenemase production among CRE**. The
272 meropenem MIC at standard inoculum poorly distinguished carbapenemase-producing isolates
273 (CP-CRE or hyper-CRE) from PD-CRE (Fig. 6*A*), making it an inefficient metric to trigger
274 carbapenemase testing. By contrast, the clear phenotypic differences across inocula between
275 these groups led us to investigate a diagnostic approach based on the IE. We quantified the IE
276 by dividing the MIC at high inoculum (1.3×10^7 CFU/mL) by the MIC at standard inoculum ($4 \times$
277 10^5 CFU/mL) for each isolate (Fig. 6*B*). CRE exhibited distinct meropenem IEs depending on
278 their mechanism of resistance, with carbapenemase-producing isolates (CP-CRE and hyper-
279 CRE) exhibiting a much more prominent IE, with a median increase in MIC of 10-fold, compared

280 to a 1.2-fold increase for PD-CRE (Fig. 6*B*). To quantify binary classification distinguishing
281 carbapenemase-producing isolates from porin-deficient ones, we generated receiver operating
282 characteristic (ROC) curves using meropenem MIC (Fig. 6C) or IE (Fig. 6D) threshold values.
283 The area under each ROC curve (AUC) represents an aggregate measure of classification
284 accuracy in distinguishing isolates by resistance mechanism across all possible binary
285 thresholds of the metric being plotted. The meropenem IE distinguished carbapenemase-
286 producing CRE from PD-CRE better than meropenem MIC at standard inoculum, illustrated by a
287 ROC AUC of 0.99 for the meropenem IE versus 0.69 for meropenem MIC. To efficiently identify
288 CRE that might carry carbapenemases, precise IE threshold values can be chosen to optimize
289 sensitivity (detecting carbapenemases) while maintaining reasonable specificity (red points in
290 Fig. 6*D*). At one specific IE threshold of 2.53-fold, the sensitivity and specificity of distinguishing
291 carbapenemase-encoding isolates from PD-CRE was 95.2% and 95.7%, respectively. The
292 meropenem IE was greater in CP-CRE (median 16-fold MIC increase) than hyper-CRE (median
293 5.3-fold), and it also distinguished these two groups (Fig. 6*B* and 6*D*; ROC AUC 0.89).
294
295 **Comparing ertapenem and meropenem MICs gives insights into resistance mechanisms.**
296 The meropenem IE effectively distinguished carbapenemase-producing isolates from PD-CRE,
297 but it is not a standard workflow in clinical laboratories, which typically only perform AST at
298 standard inocula. To overcome this limitation, since the periplasmic permeability of ertapenem is
299 typically more impacted by porin mutations than that of meropenem (6, 7, 61, 62), we
300 hypothesized that comparing ertapenem and meropenem MICs, even at a single inoculum,
301 might also distinguish CRE by mechanism of resistance. We thus measured ertapenem MICs
302 across a broad inoculum range for 85 of the 110 CRE isolates (Fig. 7). The ratio of ertapenem
303 MIC to meropenem MIC better separated carbapenemase producing isolates (CP-CRE or
304 hyper-CRE) from PD-CRE (Fig. 7*C*) than either the ertapenem or meropenem MIC alone (Fig. 7
305 *A* and *B*). At standard inoculum, meropenem MIC performed poorly at distinguishing

306 carbapenemase producers (CP-CRE or hyper-CRE) from PD-CRE (ROC AUC 0.559), whereas
307 ertapenem MIC performed slightly better (ROC AUC 0.696; Fig. 7*D*). By contrast, the
308 ertapenem-to-meropenem MIC ratio yielded a ROC AUC of 0.941, with a 90% sensitivity and
309 84% specificity at a ratio cutoff of 7.63 for detecting carbapenemase content. At low inoculum,
310 ertapenem MIC performed comparably to this ratio at identifying carbapenemase producing
311 isolates, while at high inoculum, meropenem MIC performed comparably. However, at the
312 standard CLSI inoculum, neither performed as well as the MIC ratio.
313
314 **Discussion**
315
316 Our systematic investigations revealed that the *in vitro* carbapenem inoculum effect (IE) is not
317 determined by the mechanism of action of the antibiotic, but rather by the mechanism of
318 resistance of the isolate. In *E. coli* K-12, we showed that expression of any carbapenemase is
319 sufficient to produce a carbapenem IE (Fig. 1). We confirmed this result in a collection of 106
320 clinical CRE isolates, in which all CP-CRE exhibited a clear IE, whereas PD-CRE did not (Fig. 2,
321 Supplementary Fig. S2, and Table S3). Isolates with both mechanisms of resistance (hyper-
322 CRE) tended to have higher MICs and also exhibited an IE, although smaller in magnitude than
323 CP-CRE (Fig. 2*C* and Fig. 6*B*). Although a link between β-lactamase expression and the β-
324 lactam IE has been long suspected, the link has not previously been established this clearly: in
325 *S. aureus*, for instance, the mere presence of a β-lactamase is insufficient to predict a cefazolin
326 IE (43-45). Indeed, *S. aureus* strains encoding the same family of *blaZ* β-lactamase may or may
327 not exhibit a cefazolin IE, without obvious explanation after considerable study (43, 44), despite
328 the absence of a periplasm that simplifies the system (63). Here, aided by the relatively limited
329 and well-defined subset of β-lactamases that hydrolyze carbapenems, we were able to establish
330 an obvious dichotomy in which carbapenemase content predicts a meropenem IE in the
331 Enterobacterales.

332 These findings have important mechanistic, clinical, and diagnostic implications.
333 Mechanistically, all carbapenemases imparted a clear inoculum effect despite limited sequence
334 or structural similarity across the four Ambler classes. Furthermore, we observed a consistently
335 biphasic dependence of CP-CRE MIC on inoculum (Fig. 1, Fig. 2 and Supplementary Fig. S2):
336 at the lowest inocula tested, carbapenemases conferred a relatively constant degree of
337 resistance, whereas above a certain threshold, resistance of the population increased with cell
338 density, by up to 100-fold at the highest cell densities compared with the standard inoculum
339 (Fig. 1B, Fig. 2A). This is consistent with a model where carbapenemases provide cell-
340 autonomous resistance at low density but increasing population-level resistance as cell density
341 increases, such that the collective production of carbapenemases may become the dominant
342 determinant of resistance at high enough cell density. Since the IE is only observed with
343 carbapenemase producers and not PD-CRE, one intriguing possibility is that carbapenemases
344 serve as a “common good” in bacterial populations above a certain density threshold, as has
345 been proposed for other secreted enzymes, including other β-lactamases (37-39, 64). One
346 recent study of bacterial communities in which some members produced OXA-48 supports this
347 model (40).
348 Our supernatant transfer experiments (Fig. 4, Supplementary Figs. S4 and S5) support
349 this “common good” model but add complexity: carbapenemase release into the media is
350 greatly enhanced upon antibiotic treatment, functionally consistent with antibiotic-mediated
351 altruistic cell death, a phenomenon often discussed (65) but seldom confirmed outside of
352 artificially engineered systems (66). This finding is consistent with a recent preprint proposing a
353 model whereby AmpC-mediated cefotaxime hydrolysis may accelerate after cell lysis (67).
354 Further studies are needed to more completely characterize this mechanism, including whether
355 carbapenemase activity is released as free enzyme or enclosed in outer membrane vesicles
356 (68-70), and whether or how its release is regulated upon antibiotic exposure, versus passively
357 spilled by cell death. The enhanced release of carbapenemase activity by apramycin

358 (Supplementary Fig. S5), an unrelated antibiotic class that inhibits ribosome function, implies
359 that new protein synthesis is not required for release, perhaps favoring a passive release
360 mechanism. Exploration of the implications of this shared activity for emergent resistance in CP-
361 CRE populations compared with individual cells, both in laboratory culture and in structured and
362 mixed bacterial communities that better reflect natural environments, is also warranted.

363 Notably, PD-CRE isolates expressing extended spectrum β-lactamases (ESBLs), which
364 often contribute to carbapenem resistance, did not exhibit an appreciable carbapenem IE (Fig.
365 2*B* and Supplementary Fig. S2*B*), nor did several meropenem-susceptible isolates, including
366 some encoding an ESBL and other randomly selected non-ESBL-producers (Supplementary
367 Fig. S2*D*). Consistent with these observations, transformation of the common ESBL gene bla_{CTX-}
368 $_{M-15}$ did not confer a meropenem IE upon *E. coli* K-12 (Supplementary Fig. S1). Together, these
369 findings indicate that the slow rates of carbapenem hydrolysis by ESBL enzymes were
370 insufficient to benefit neighboring bacteria even at high culture density. The lower prevalence of
371 carbapenemases compared to other β-lactamases may explain why the IE is reported less
372 frequently for carbapenems than for other β-lactams (31), but this might change as
373 carbapenemases continue to spread globally.

374 The clinical implications of the inoculum effect have been uncertain because the relevant
375 bacterial cell densities during most infections *in vivo* are generally below those used for AST
376 (61, 64). For instance, in bacteremia, the infectious burden of ≤10 CFU/mL is several orders of
377 magnitude below that at which the inoculum begins to affect MIC in our assays (71, 72).
378 Nevertheless, in *Staphylococcus aureus* bacteremia, a cefazolin IE has been associated with
379 treatment failure and higher mortality (73, 74). Our findings suggest that at the low inoculum
380 present in bacteremia, PD-CRE (Fig. 2*B*, Supplementary Fig. S2*B*) exhibit higher carbapenem
381 MICs than CP-CRE (Fig. 2*A*, Supplementary Fig. S2*A*), yet clinical outcomes are similar or
382 worse for CP-CRE bacteremia (75, 76). It is possible that the increased resistance of PD-CRE
383 at physiologically relevant bacterial densities may be offset by fitness costs associated with

384 reduced permeability. Further research is needed to fully understand the relationship between
385 MIC, IE, resistance mechanisms, and clinical outcomes. Intriguing recent studies found that
386 isolates encoding carbapenemases and displaying a large IE require higher meropenem
387 concentration to prevent emergence of highly resistant mutants (39, 59). Since we observed the
388 largest IE among CP-CRE with intact porins, perhaps these extremely resistant escape mutants
389 reflect *de novo* porin mutations, creating hyper-CRE.

390 Diagnostically, accurate recognition of carbapenem resistance is crucial for infection
391 control strategies and patient care. Our results confirmed prior reports that minor differences in
392 inoculum can lead to categorical changes in susceptibility interpretation (48), particularly
393 impacting the detection of CP-CRE, the isolates that carry the greatest implications for infection
394 control. Even though the isolates we examined were selected because they tested as CRE,
395 many changed classification within the CLSI-acceptable inoculum range and even appeared
396 susceptible (Fig. 5), suggesting that clinical algorithms may miss some isolates encoding
397 carbapenemases (77). In fact, meropenem and ertapenem MICs of all CP-CRE isolates varied
398 significantly in this range. Our observations in *E. coli* K-12 (Fig. 1) reveal that strains harboring
399 OXA-48, CMY-10, or even KPC might only test resistant if they possess other factors, such as
400 reduced antibiotic permeability. The clinical significance of isolates that express a
401 carbapenemase but test susceptible in the standard inoculum range is uncertain and requires
402 further study. Nonetheless, caution is generally advised when using carbapenems in infections
403 with carbapenemase-producers, even if they test susceptible, due to the lack of evidence
404 supporting clinical outcomes in these cases (24, 78). Our findings in a set of isolates selected
405 for meropenem nonsusceptibility suggest that other carbapenemase producers may have been
406 missed by standard diagnostic workflows, which could contribute to poor patient outcomes and
407 the global spread of carbapenem resistance.

408 The strong correlation between the carbapenem IE and genotype (Fig. 2) allows for
409 improved predictions about resistance mechanisms from phenotypic testing. Although MICs

410 were not designed to identify resistance mechanisms, carbapenem MICs are routinely used to
411 prioritize isolates for molecular carbapenemase testing (79), though different MIC thresholds for
412 testing are recommended in the US (24) and Europe (27). Our study revealed that the
413 meropenem IE is a more sensitive and specific indicator of carbapenemase production in CRE
414 than MIC at any single inoculum (Fig. 6). Although measuring the meropenem IE would require
415 additional testing workflows, which are likely impractical in overworked clinical microbiology
416 laboratories, we also found that integrating ertapenem and meropenem MICs from routinely
417 generated AST data at standard inoculum can inform the likelihood of carbapenemase
418 production (Fig. 6). The ratio of ertapenem to meropenem MIC predicts resistance mechanism
419 better than either MIC alone, perhaps because the periplasmic permeability of ertapenem is
420 more strongly affected by porin disruption than that of meropenem (6, 61, 62), while both are
421 hydrolyzed at similar rates by carbapenemases (80, 81). Specifically, CRE with a high ratio of
422 ertapenem to meropenem MIC (more resistant to ertapenem) are likely to be PD-CRE, whereas
423 those with a low MIC ratio (similar resistance to both) are likely to have wild-type porins, and
424 thus likely require a carbapenemase to achieve carbapenem resistance. As with the IE, hyper-
425 CRE appear to fall in between, and tend to have higher MICs at any inoculum. Since the IE
426 impacts both ertapenem and meropenem MICs similarly (Fig. 5), this ratio predicts resistance
427 mechanism independent of inoculum, an advantage over either ertapenem or meropenem MICs
428 alone (Fig. 7).

429 We propose that this ertapenem to meropenem MIC ratio might therefore be better
430 suited than either MIC alone to prioritize isolates for molecular or enzymatic carbapenemase
431 detection (20, 82, 83). Recognizing that MIC thresholds for susceptibility may miss
432 carbapenemase-producing isolates, EUCAST recommends a 16-fold lower MIC threshold for
433 carbapenemase screening than their clinical susceptibility breakpoint (27). This lower threshold
434 will lead to more sensitive carbapenemase detection, but at lower specificity, requiring far more
435 testing. If validated in further studies at this lower MIC threshold, the ertapenem to meropenem

436 MIC ratio may enable optimization of strain selection for molecular carbapenemase testing,
437 increasing screening efficiency below the susceptibility breakpoint. By more efficiently
438 identifying carbapenemase-producing isolates, our work could facilitate implementation of
439 recent advances showing that molecular detection of carbapenemases can accelerate
440 administration of targeted therapies, such as β-lactamase inhibitor combinations (84), and
441 decrease mortality (22). Moreover, this phenotypic approach could detect strains producing rare
442 carbapenemases, such as SME or CMY-10, that are not routinely included in testing panels,
443 akin to the Carba NP (24, 85) and mCIM (21) methods. This finding, in addition to its therapeutic
444 value, could serve as a surveillance tool for the emergence and evolution of carbapenemases
445 (86). This work does not aim to replace well-validated clinical carbapenemase detection
446 methods. Instead, it illustrates how biological phenomena can offer further insights into
447 resistance mechanism in a clinical context, perhaps improving the efficiency of deployment of
448 existing carbapenemase detection assays.

449 This study has several limitations and areas requiring further study. First, the clinical
450 isolates analyzed were mostly chosen because they tested carbapenem resistant, limiting the
451 ability to determine the frequency of CP-CRE isolates that may be missed by routine testing due
452 to the IE. Further studies are needed to assess the performance of our proposed assays on
453 isolates with MICs at or below the susceptible breakpoint. Second, automated AST instruments
454 may not provide precise MIC measurements for ertapenem and meropenem outside a narrow
455 range around the susceptibility breakpoints, requiring further investigation into the practical
456 utility of these lower-resolution MIC estimates in screening for CP-CRE. Third, while we found
457 carbapenemases sufficient to confer a carbapenem IE, other factors such as heteroresistance,
458 metabolic factors, quorum sensing, and target distribution may also contribute in some isolates
459 or circumstances. However, carbapenemases predictably cause a robust IE without requiring
460 other factors. Our findings reveal a clear pattern: as the inoculum increases above a certain
461 threshold, we observe a steady linear rise in MIC (Fig. 2 and Supplementary Fig. S2). This

462 pattern is less consistent with heteroresistance, an alternative explanation for the IE whereby a
463 rare resistant subpopulation emerges from larger inocula. With heteroresistance, one would
464 expect a sudden step-like increase in MIC as inoculum rises, as opposed to the gradual MIC
465 increase that we consistently observed. Lastly, this study did not explore the basis of the IE for
466 other antibiotics, nor how other processes like antibiotic efflux may contribute to carbapenem
467 resistance. However, the consistent relationship between resistance mechanism and the IE for
468 carbapenems, where hydrolytic enzymes and other resistance mechanisms are relatively few
469 and well characterized, may offer a useful general model for one major mechanism underlying
470 the IE. Future studies could involve pairing these detailed phenotypic data and genotypic
471 information (Supplementary Table S3) with precise enzymological parameters and direct
472 quantification of enzymes and metabolites in fractionated cytoplasm, periplasm, vesicles, and
473 supernatant in order to enhance and experimentally test existing quantitative models (41, 64).
474 This would enable a more comprehensive description of how different mechanisms of antibiotic
475 resistance impact the growth of bacterial populations when exposed to antibiotic selection.
476 Antibiotic resistance often reflects several molecular processes acting in concert.
477 Carbapenemase content and porin status together explained the levels of carbapenem
478 resistance in most isolates we tested. Some genera like *Klebsiella* appear to exhibit higher
479 carbapenem MICs than others like *Citrobacter* even when they encode the same
480 carbapenemase-containing plasmid (14). This may reflect baseline differences in permeability
481 by phylogenetic grouping, which is consistent with general trends in the ertapenem-to-
482 meropenem MIC ratio across species in our collection (Supplementary Table S3). However,
483 some isolates were outliers, exhibiting unexpectedly high resistance or inoculum-dependent
484 patterns that suggest uncharacterized elements contribute to their carbapenem resistance. In
485 some cases, these unexpected behaviors were clues to simple errors in sample workup. For
486 instance, in one isolate (MGH4), the deposited genome assembly did not reveal a
487 carbapenemase (12) but it exhibited a strong meropenem MIC suggestive of a CP-CRE. We

488 performed PCR and Sanger sequencing, revealing that MGH4 harbored a KPC, consistent with
489 the IE. The plasmid was likely lost during strain passaging prior to whole genome sequencing.
490 Detailed IE phenotype also allowed insights into porin polymorphisms of uncertain significance,
491 which are a frequent challenge for genotypic prediction of β-lactam resistance (80, 82, 83, 87-
492 91). Given the differential impact of porin mutations on each carbapenem, the ertapenem-to-
493 meropenem MIC ratio may offer a phenotypic reflection of porin function. This simple parameter,
494 derived from values already routinely measured, may thus be useful in assessing resistance
495 mechanisms to carbapenems and other β-lactams. Even with the insight generated in this study,
496 we observed that some of our CRE isolates had unexplained levels or patterns of carbapenem
497 resistance across a broad range of inocula. Such outliers underscore remaining gaps in our
498 knowledge and motivate further investigations to characterize unknown processes leading to
499 high-level resistance, which might be encountered more frequently as CRE continue to spread
500 globally.
501
502
503 **Methods:**
504
505 **Bacterial isolates.** Most of the bacterial strains tested in this paper were collected from three
506 major Boston-area hospitals (Beth Israel Deaconess Medical Center, BIDMC; Brigham and
507 Women's Hospital, BWH; Massachusetts General Hospital, MGH) and the University of
508 California Irvine School of Medicine (UCI) as part of separate studies (12, 14), and approved by
509 each hospital and the Broad Institute, adjudicated under Broad Office of Research Subject
510 Protections (ORSP) designation ORSP-1833; additional IRB approval was granted by the
511 Massachusetts Institute of Technology Committee on the Use of Humans as Experimental
512 Subjects. Collection of additional isolates from the MGH and BWH clinical microbiology
513 laboratories was approved by the Partners Health Care Institutional Review Board (IRB) under

514 protocol 2015P002215. Additional isolates were acquired from existing publicly available
515 collections through the Wadsworth Center of the New York State Department of Health
516 (NYSDOH), the FDA-CDC Antimicrobial Resistance Isolate Bank (CDC, Atlanta, GA), and the
517 American Type Culture Collection (ATCC) (Manassas, VA).
518
519 **Cloning of carbapenemases**. *bla*$_{KPC-3}$, *bla*$_{SME-2}$, *bla*$_{IMP-4}$, *bla*$_{NDM-1}$, *bla*$_{VIM-27}$, and *bla*$_{OXA-48}$ along
520 with their native promoters were cloned by Gibson assembly from isolates in our existing CRE
521 collection (12, 14) encoding these carbapenemases into a pBAD33 plasmid which encodes
522 chloramphenicol resistance (*SI Appendix*, Table S1). *bla*$_{CMY-10}$ with its native promoter was
523 synthesized in the same pBAD33 vector backbone by GeneWiz (Suzhou, China), since we did
524 not have a clinical isolate expressing this enzyme.
525
526 **Broad-range broth microdilution.** Our MIC assays were adapted from previously published
527 broth microdilution methods (23, 92). See Supplemental Methods for details on setup of the
528 broad inoculum range in this assay format.
529
530 **Supernatant transfer experiments.** The producer strain (see Fig. 4*A* for schematic) was grown
531 to logarithmic phase (OD_{600} 0.1 – 0.4) in MHB (Sigma, 70192), then back-diluted to a target
532 inoculum of 1.3e x 10^7 CFU/mL in 16 mL and incubated with or without antibiotic for 2 hours at
533 37ºC on a shaking platform (250 rpm). Meropenem treatments were at 1/2x and apramycin
534 treatments were at 4x the standard-inoculum MIC of each producer strain. After incubation,
535 cultures were pelleted at 5000xg for 5 minutes at 4ºC. Supernatants were syringe-filtered with
536 0.2 μm cellulose acetate filters (Cytiva, 6901-2502) to remove remaining producer cells,
537 transferred to 10 kDa Amicon Ultra-15 centrifugal filter units (Millipore Sigma, UFC901025) pre-
538 rinsed in phosphate-buffered saline (PBS), and spun at 5000xg at 4ºC until concentrated to 1/10
539 of the original volume. Concentrated supernatants from meropenem-treated conditions were

540 washed 3 times to remove meropenem (which would otherwise interfere with the reporter MIC
541 assay) by adding PBS to the original volume and re-concentrating by 10x, then underwent a
542 final MHB wash. This 10x-concentrated conditioned media was then added at a final 1x
543 concentration to a standard meropenem broth microdilution MIC assay, with the reporter strain
544 at standard inoculum (4 x 10^5 CFU/mL) in MHB (Difco, 275730). For experiments with
545 meropenem-treated producer strains (Fig. 4, Supplementary Fig. S3*A*), the reporter strain was
546 *E. coli* K-12 MG1655; for apramycin experiments (Supplementary Figs. S3*B* and S4), the
547 reporter strain was *E. coli* K-12 MG1655 transformed with an empty vector marked with an
548 apramycin resistance gene (pBAD33-AprR), to eliminate the need for wash steps to remove the
549 antibiotic from the producer supernatant. Vaborbactam (Cayman Chemical, 23962) was added
550 at a final concentration of 8 μg/mL in select reporter MIC assays. See Supplemental Methods
551 for further details.
552
553 **Data analysis.** MIC replicates were imported to GraphPad Prism v 9.5.1 (GraphPad Software
554 Inc., San Diego, CA); MIC vs inoculum plots (Figs. 1, 2, and 3, and Supplementary Fig. S1)
555 depict mean and standard errors of the mean. To analyze the meropenem IE, the ratio of mean
556 MIC at 1.3 × 10^7 CFU/mL to 4 × 10^5 CFU/mL was calculated. The IE of the 47 porin deficient
557 CRE strains were compared to the 63 carbapenemase-encoding CRE strains using a non-
558 parametric test (Mann-Whitney for comparisons between two groups or Kruskal-Wallis for
559 analysis of more than two groups) in GraphPad Prism. Statistical analysis comparing PD-CRE
560 isolates versus CP-CRE and hyper-CRE isolate at low, standard, and high inocula for
561 ertapenem, meropenem, and ertapenem-to-meropenem MIC ratio was conducted using Mann-
562 Whitney tests. All receiver operating characteristic (ROC) curves and analysis were constructed
563 using GraphPad Prism.
564

565 **Gene editing to disrupt OmpK36 in *Klebsiella pneumoniae* isolate RB582.** A CRISPR-Cas9
566 cytidine base-editing system (56) was used to introduce an early termination codon in the intact
567 *ompK36* of isolate RB582. See Supplemental Methods for further details.
568

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573
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Figures

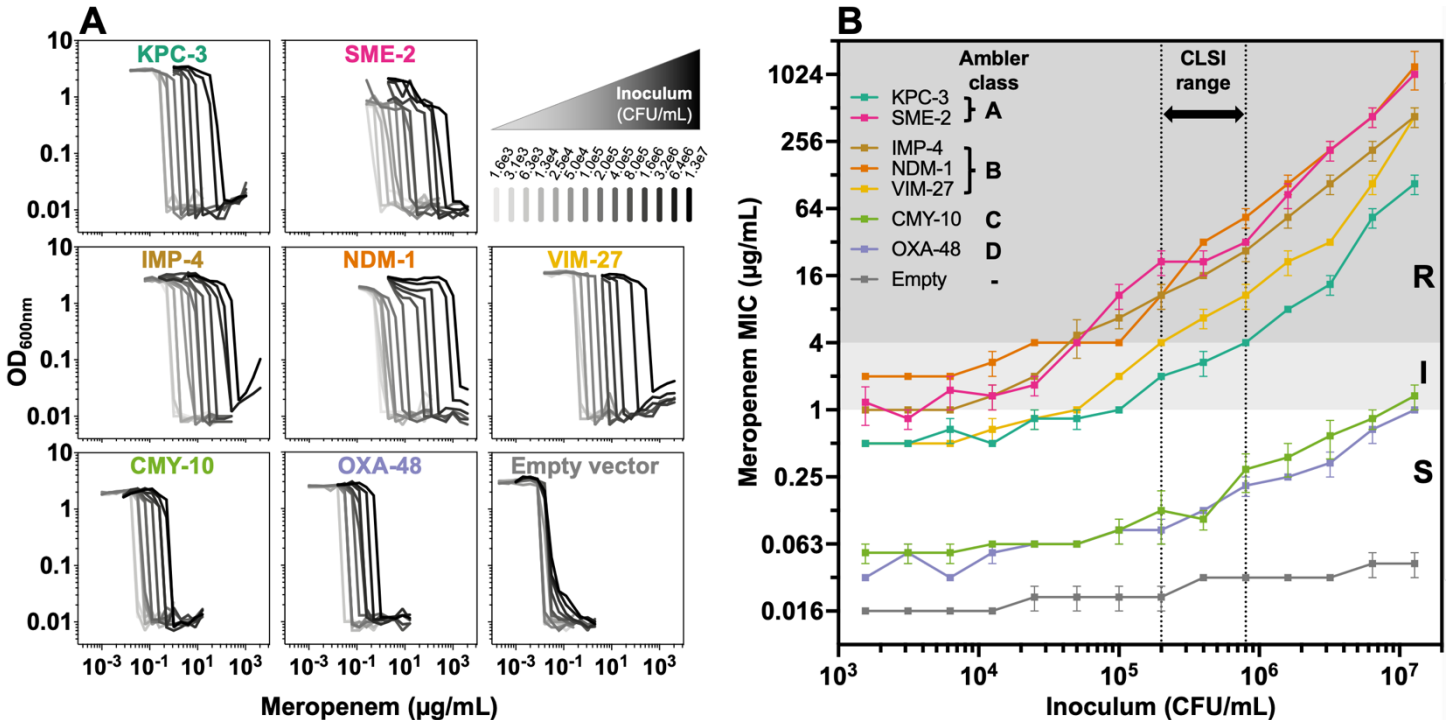

Fig. 1. All carbapenemases impart a meropenem inoculum effect when expressed in *E. coli K12*. (A) Plots of optical density (OD) at 600 nm (y-axis) after overnight incubation with varying meropenem concentrations (x-axis) across a broad range of starting inocula (grayscale per top right panel) for *E. coli* K-12 transformed with each indicated carbapenemase. **(B)** Meropenem minimum inhibitory concentration (MIC) as a function of bacterial inoculum, colored by transformed carbapenemase. Each MIC point is the mean of three replicates (error bars = standard error of the mean). Vertical dotted lines represent the CLSI-acceptable inoculum range (2 to 8 x 10^5 CFU/mL). The background is shaded by CLSI meropenem susceptibility breakpoints (S = susceptible, white; I = intermediate, light gray; R = resistant, gray).

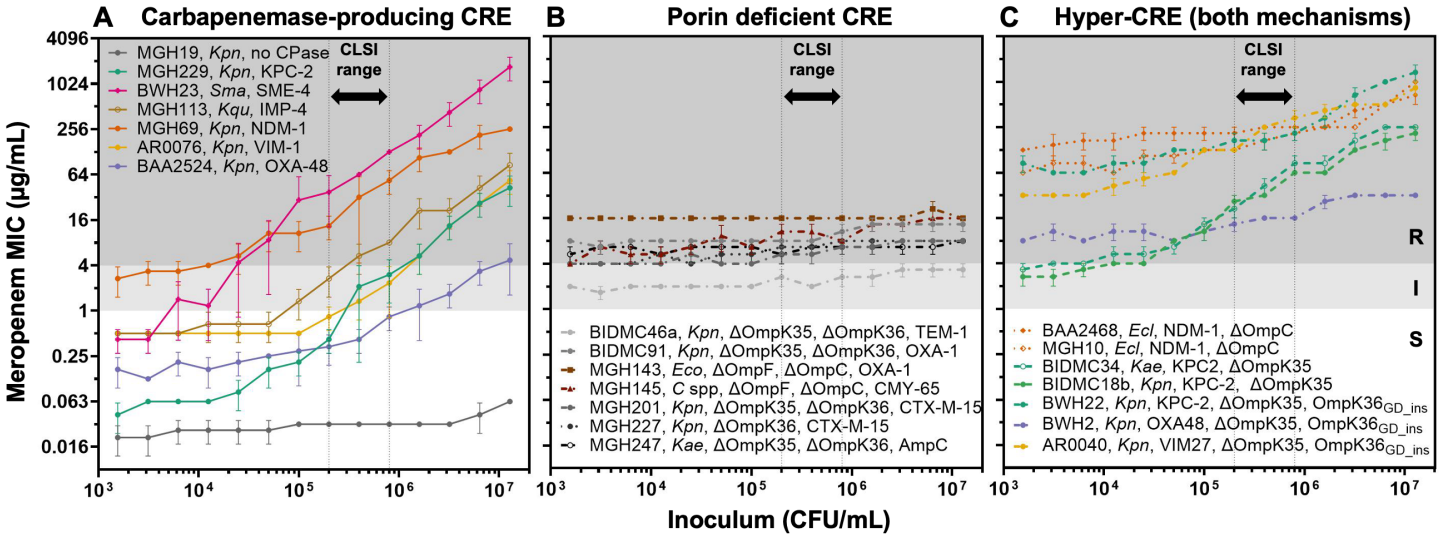

Fig. 2. Select CRE exhibit different patterns of carbapenem inoculum effect depending on resistance mechanism. Meropenem MICs from broth microdilution assays (y-axis) across a broad range of starting inocula (x-axis) of select clinical CRE isolates encoding **(A)** a carbapenemase of each major type, **(B)** porin deficiency, or **(C)** both, with species and relevant genotype as indicated. One susceptible isolate (MGH19, gray) is also shown in panel **(A)**. Each MIC point is the mean of three replicates (error bars = standard error of the mean). *C spp* = *Citrobacter* spp, *Ecl* = *Enterobacter cloacae*, *Eco* = *Escherichia coli*, *Kae* = *Klebsiella aerogenes*, *Kpn* = *Klebsiella pneumoniae*, *Kqu* = *Klebsiella quaispneumoniae*, *Sma* = *Serratia marcescens*. CPase = carbapenemase. Vertical dotted lines reflect the CLSI-recommended inoculum range, background shading reflects CLSI breakpoints, and line color reflects carbapenemase content, all as in Fig. 1; dashed lines indicate porin deficiency.

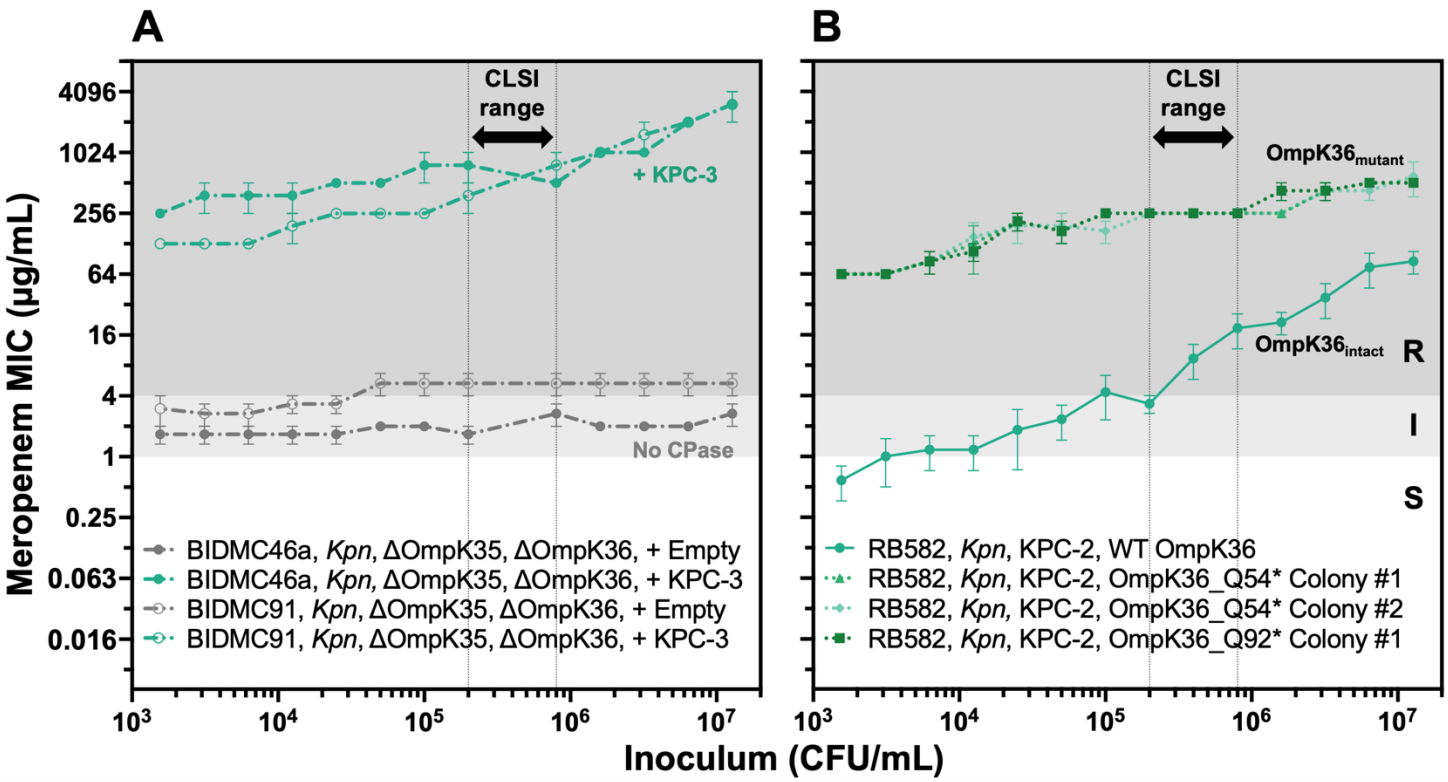

Fig. 3. Carbapenemase production and porin deficiency together create a hyper-CRE phenotype. Broad-range meropenem broth microdilution assays for **(A)** two clinical PD-CRE isolates before and after transformation with pBAD33_KPC-3 and **(B)** a clinical CP-CRE isolate and three derivative colonies in which OmpK36 was disrupted by CRISPR-Cas9 based gene editing to create a premature stop at one of two glutamines as indicated. The parental KPC-expressing isolate (RB582) is shown as a solid line with green markers. Each MIC point is the mean of three replicates (error bars = standard error of the mean). Vertical dotted lines reflect the CLSI-recommended inoculum range, background shading reflects CLSI breakpoints, and line color reflects carbapenemase content as in Fig. 1; dashed lines indicate porin deficiency as in Fig. 2. *Kpn* = *Klebsiella pneumoniae.*

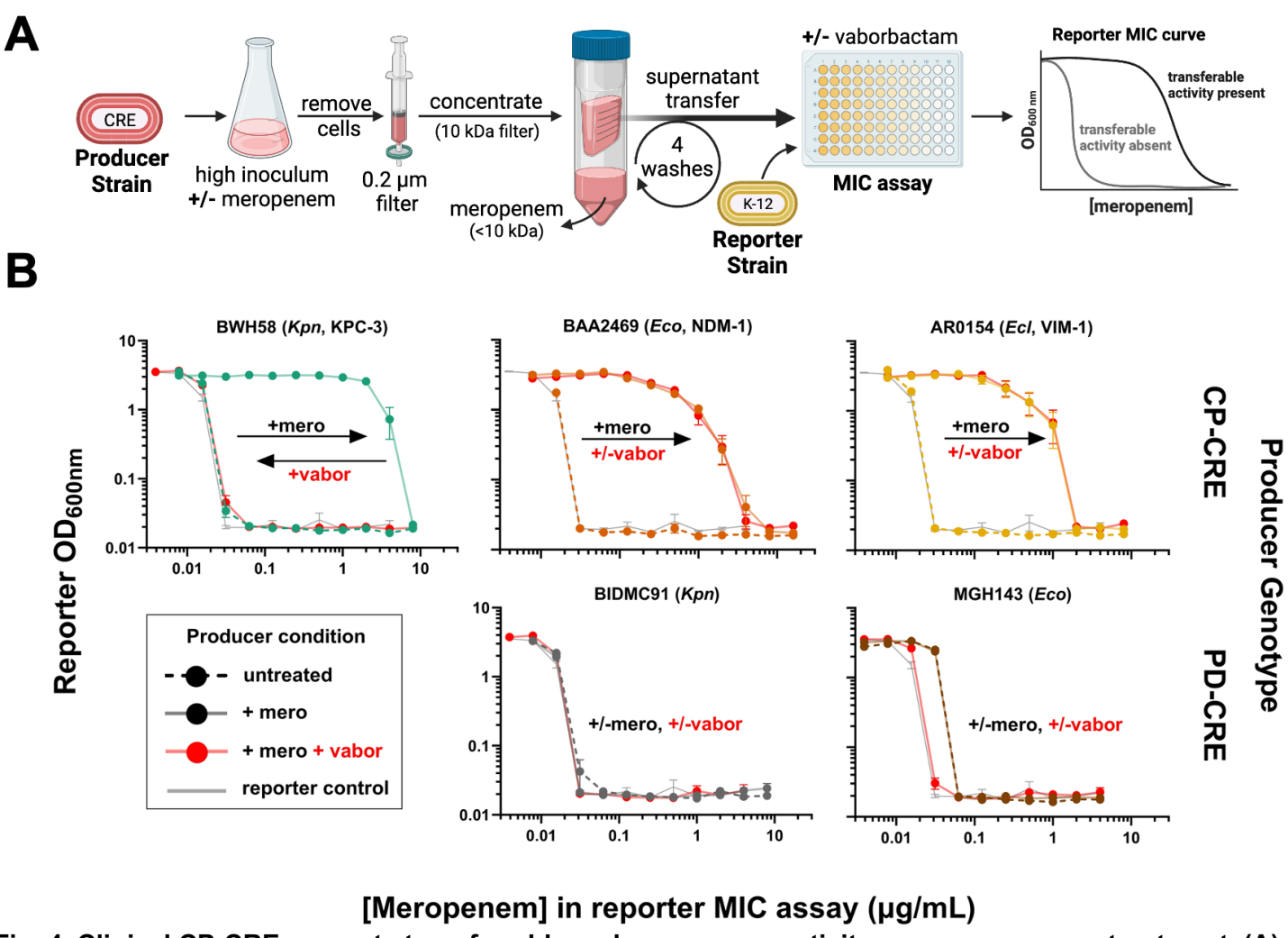

Fig. 4. Clinical CP-CRE generate transferrable carbapenemase activity upon meropenem treatment. (A) Supernatant transfer experiment workflow: CP-CRE or PD-CRE isolates ("producer strains") were grown to high inoculum, then exposed to media with or without antibiotic for 2 hours. Culture supernatants were filtered, washed to remove antibiotic, concentrated, and added to meropenem MIC assays for susceptible *E. coli* K-12 reporter strains to assess for transferrable protection. Mock data on the right shows expected results from reporter MIC assays if the supernatant contains protective activity (black) or not (gray). **(B)** Plots of reporter *E. coli* K-12 meropenem MIC assays, supplemented with supernatants from producer strains treated with (solid lines) or without (dashed lines) meropenem prior to supernatant transfer. CP-CRE producers are colored by carbapenemase content as in Fig. 1; gray curves = MIC of reporter strain alone; red curves = vaborbactam added to reporter MIC assay. Data from untreated samples and treated PD-CRE producers are the mean of three replicates of reporter MIC assays from a single producer treatment. All other data are the mean of nine replicates, three reporter MIC assays from each of three producer treatment replicates (error bars = standard error of the mean). *Ecl* = *Enterobacter cloacae*, *Eco* = *Escherichia coli*, *Kpn* = *Klebsiella pneumoniae;* mero = meropenem, vabor = vaborbactam. Isolates are colored by carbapenemase content as in Fig. 1.

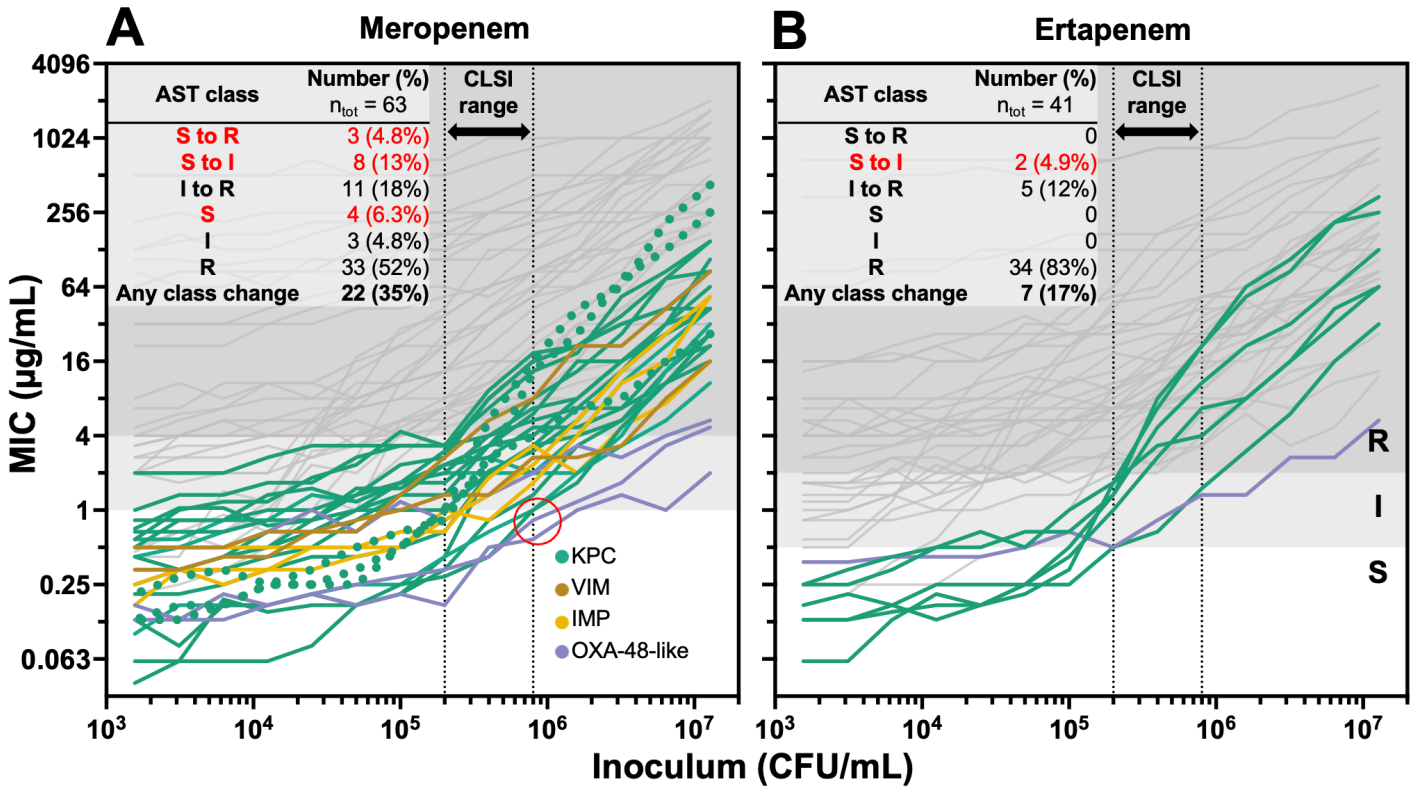

Fig 5. Many carbapenemase-producing isolates change AST classification in the CLSI range. Broad-range broth microdilution assays for **(A)** 63 carbapenemase-producing clinical CRE isolates (36 CP-CRE and 27 hyper-CRE) treated with meropenem and **(B)** 41 carbapenemase-producing clinical CRE isolates (30 CP-CRE and 11 hyper-CRE) treated with ertapenem. Each MIC measurement was the mean of three replicates; error bars were omitted for simplicity. Isolates that tested anything other than resistant at some point in the CLSI-recommended inoculum range are colored by carbapenemase content; those that tested resistant throughout the CLSI range are in gray. Inset table shows the number and percentage of isolates by AST class across the CLSI inoculum range; red text indicates isolates that tested susceptible at some point within the CLSI range. The red circle highlights 4 isolates that tested fully meropenem-susceptible throughout the CLSI range; dotted lines indicate 3 isolates whose meropenem susceptibility category changed from susceptible to resistant in the CLSI range.

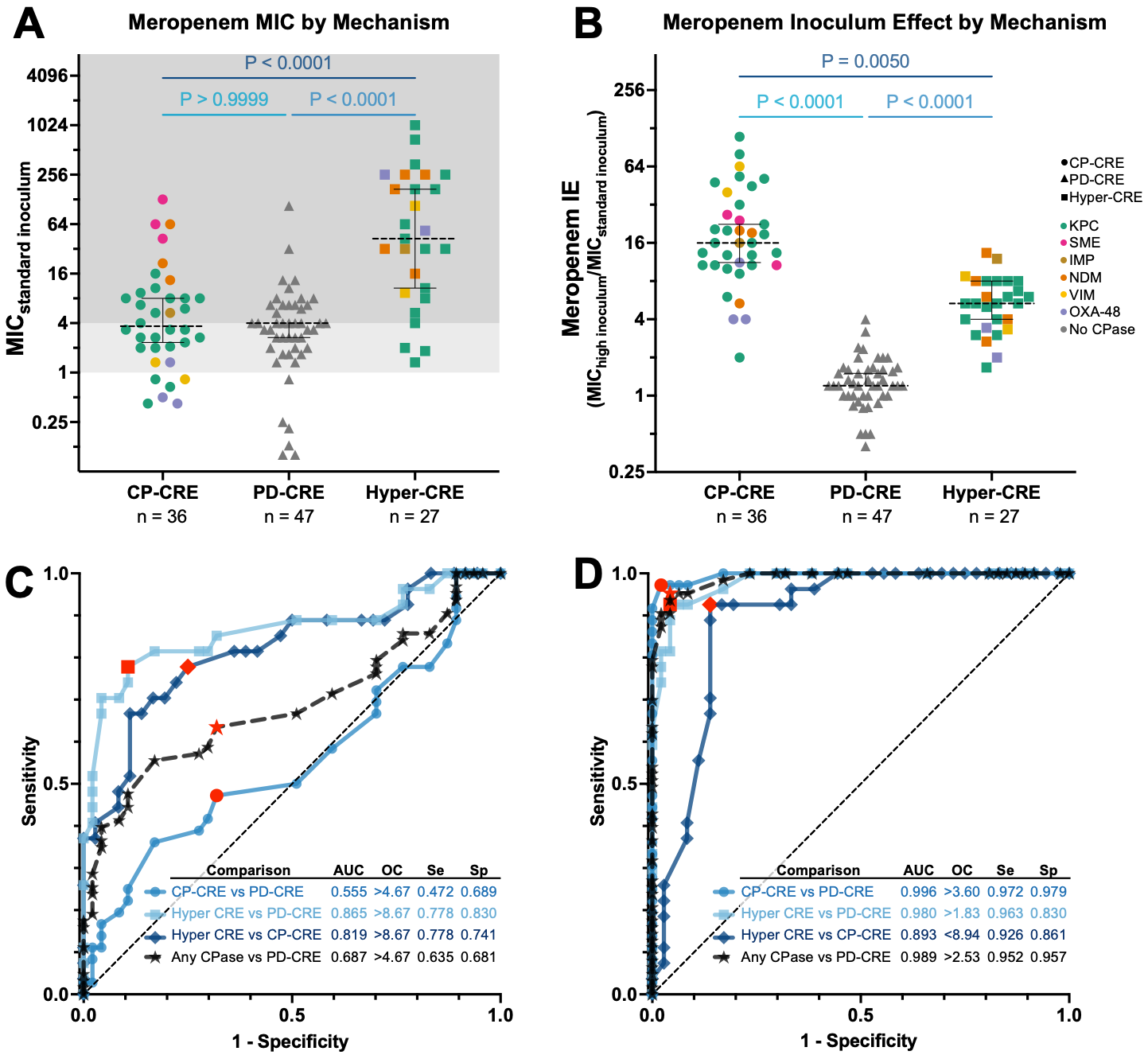

Fig. 6. Meropenem inoculum effect robustly classifies CRE isolates by resistance mechanism. (A) Meropenem MICs for 106 CRE and 4 porin-deficient CSE (PD-CSE), grouped by genotypic mechanism of carbapenem resistance: CP-CRE (circles), PD-CRE or PD-CSE (triangles), or hyper-CRE (squares). Isolates are colored by carbapenemase content as in Fig. 1. Each MIC point is the mean of three replicates. **(B)** Ratio of meropenem MIC at high (1.3 x 10^7 CFU/mL) versus standard (4.0 x 10^5 CFU/mL) inoculum for 106 CRE and 4 PD-CSE, grouped as in **(A).** P-values were obtained by conducting statistical analyses on the three groups in **(A)** and **(B)** using a Kruskal-Wallis test. **(C)** Receiver operating characteristic (ROC) curves based on the meropenem MICs at standard inoculum from **(A)** comparing the different genotypic CRE groups. Inset tabulates each ROC area under the curve (AUC), sensitivity (Se), and specificity (Sp) at an optimal cutoff (OC) threshold, indicated by the red points on the ROC curves. **(D)** ROC curves based on the meropenem inoculum effect, displayed as in **(C)**. Any CPase = either CP-CRE or hyper-CRE.

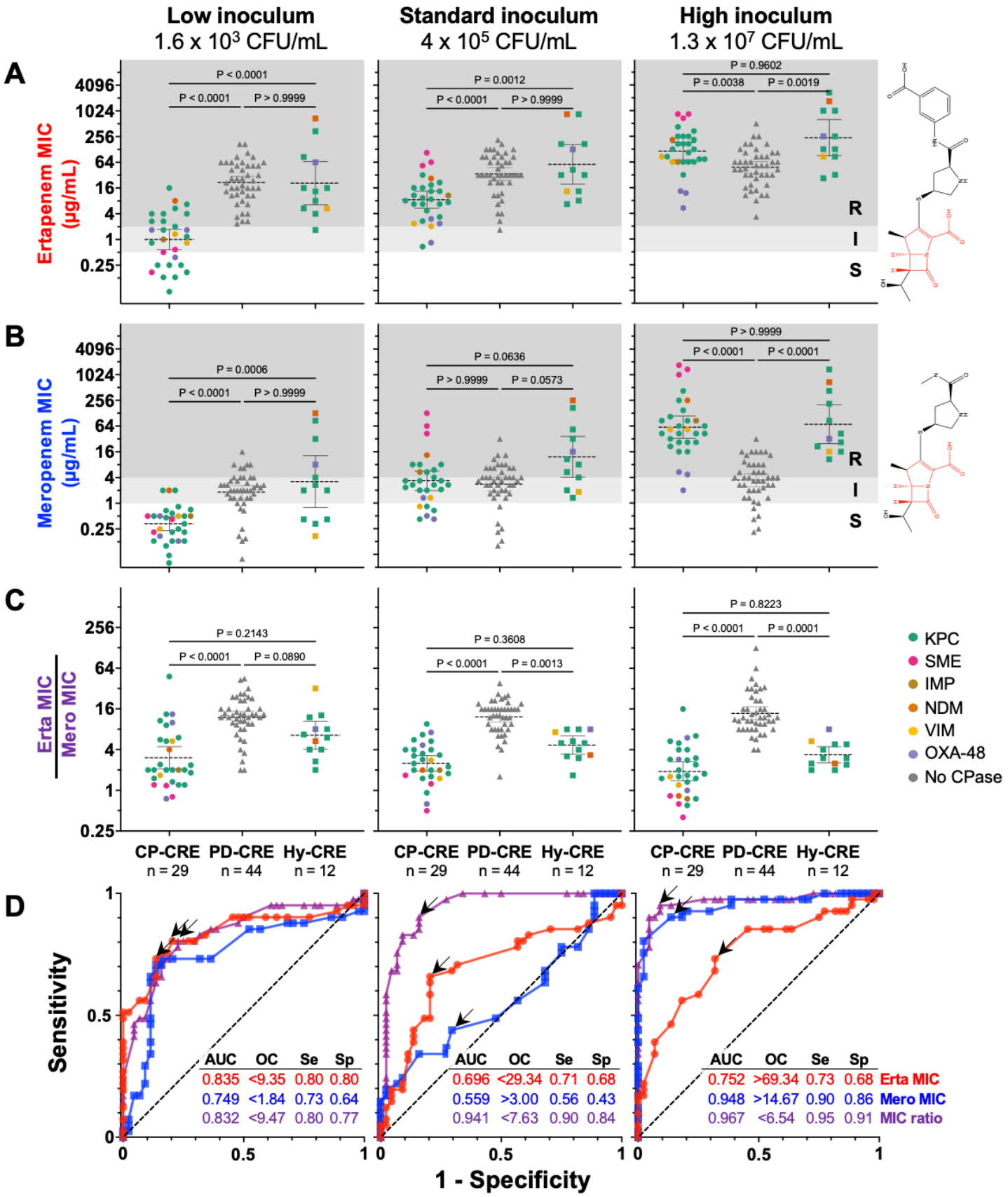

Fig. 7. Comparing ertapenem and meropenem MICs gives insight into resistance mechanism (A-C) MICs of ertapenem **(A)** or meropenem **(B)**, or the ratio of the ertapenem MIC to the meropenem MIC **(C),** are shown for 85 isolates at low, standard, and high inoculum (left, middle, and right panels respectively), grouped by resistance mechanism along the x-axis (hyper-CRE = "Hy-CRE"). Data points are colored based on

carbapenemase content as in Fig. 1. Plot backgrounds are shaded by CLSI breakpoints for ertapenem **(A**) or meropenem **(B**) as in Fig. 1. P-values reflect non-parametric Kruskal-Wallis tests for each between-group comparison. In chemical structures, the carbapenem core is red and the modifiable substituents are black. **(D)** ROC curves comparing carbapenemase producers (CP-CRE + hyper-CRE) vs PD-CRE for each metric (red = ertapenem MIC, blue = meropenem MIC, purple = MIC ratio) at low, standard, and high inoculum. Inset tabulates ROC AUC and sensitivity (Se) and specificity (Sp) at an optimal cutoff (OC, indicated by arrow on ROC curve).

REFERENCES:

1. El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. 2017. Recent updates of carbapenem antibiotics. Eur J Med Chem 131:185-195.
2. Codjoe FS, Donkor ES. 2017. Carbapenem Resistance: A Review. Med Sci (Basel) 6.
3. CDC. 2019. Antibiotic Resistance Threats in the United States, 2019. US Department of Health and Human Services C, Atlanta, GA.
4. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Group NCSP, Henderson DK, Palmore TN, Segre JA. 2012. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 4:148ra116.
5. Bush K, Bradford PA. 2020. Epidemiology of beta-Lactamase-Producing Pathogens. Clin Microbiol Rev 33.
6. Rocker A, Lacey JA, Belousoff MJ, Wilksch JJ, Strugnell RA, Davies MR, Lithgow T. 2020. Global Trends in Proteome Remodeling of the Outer Membrane Modulate Antimicrobial Permeability in Klebsiella pneumoniae. mBio 11.
7. Acosta-Gutierrez S, Ferrara L, Pathania M, Masi M, Wang J, Bodrenko I, Zahn M, Winterhalter M, Stavenger RA, Pages JM, Naismith JH, van den Berg B, Page MGP, Ceccarelli M. 2018. Getting Drugs into Gram-Negative Bacteria: Rational Rules for Permeation through General Porins. ACS Infect Dis 4:1487-1498.
8. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, Blair JMA. 2023. Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol 21:280-295.
9. Pai H, Kim J, Kim J, Lee JH, Choe KW, Gotoh N. 2001. Carbapenem resistance mechanisms in Pseudomonas aeruginosa clinical isolates. Antimicrob Agents Chemother 45:480-4.
10. Zhang Y, Li Z, He X, Ding F, Wu W, Luo Y, Fan B, Cao H. 2017. Overproduction of efflux pumps caused reduced susceptibility to carbapenem under consecutive imipenem-selected stress in Acinetobacter baumannii. Infect Drug Resist 11:457-467.
11. Schwaber MJ, Lev B, Israeli A, Solter E, Smollan G, Rubinovitch B, Shalit I, Carmeli Y, Israel Carbapenem-Resistant Enterobacteriaceae Working G. 2011. Containment of a country-wide outbreak of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals via a nationally implemented intervention. Clin Infect Dis 52:848-55.
12. Cerqueira GC, Earl AM, Ernst CM, Grad YH, Dekker JP, Feldgarden M, Chapman SB, Reis-Cunha JL, Shea TP, Young S, Zeng Q, Delaney ML, Kim D, Peterson EM, O'Brien TF, Ferraro MJ, Hooper DC, Huang SS, Kirby JE, Onderdonk AB, Birren BW, Hung DT, Cosimi LA, Wortman JR, Murphy CI, Hanage WP. 2017. Multi-institute analysis of carbapenem resistance reveals remarkable diversity, unexplained mechanisms, and limited clonal outbreaks. Proc Natl Acad Sci U S A 114:1135-1140.
13. Mathers AJ, Cox HL, Kitchel B, Bonatti H, Brassinga AK, Carroll J, Scheld WM, Hazen KC, Sifri CD. 2011. Molecular dissection of an outbreak of carbapenem-resistant enterobacteriaceae reveals Intergenus KPC carbapenemase transmission through a promiscuous plasmid. mBio 2:e00204-11.
14. Salamzade R, Manson AL, Walker BJ, Brennan-Krohn T, Worby CJ, Ma P, He LL, Shea TP, Qu J, Chapman SB, Howe W, Young SK, Wurster JI, Delaney ML, Kanjilal S, Onderdonk AB, Bittencourt CE, Gussin GM, Kim D, Peterson EM, Ferraro MJ, Hooper DC, Shenoy ES, Cuomo CA, Cosimi LA, Huang SS, Kirby JE, Pierce VM, Bhattacharyya RP, Earl AM. 2022. Inter-species geographic signatures for tracing horizontal gene transfer and long-term persistence of carbapenem resistance. Genome Med 14:37.
15. Choi U, Lee CR. 2019. Distinct Roles of Outer Membrane Porins in Antibiotic Resistance and Membrane Integrity in Escherichia coli. Front Microbiol 10:953.
16. Alva A, Sabido-Ramos A, Escalante A, Bolivar F. 2020. New insights into transport capability of sugars and its impact on growth from novel mutants of Escherichia coli. Appl Microbiol Biotechnol 104:1463-1479.
17. Andersen J, Forst SA, Zhao K, Inouye M, Delihas N. 1989. The function of micF RNA. micF RNA is a major factor in the thermal regulation of OmpF protein in Escherichia coli. J Biol Chem 264:17961-70.
18. Abellon-Ruiz J, Kaptan SS, Basle A, Claudi B, Bumann D, Kleinekathofer U, van den Berg B. 2017. Structural basis for maintenance of bacterial outer membrane lipid asymmetry. Nat Microbiol 2:1616-1623.

19. Goodman KE, Simner PJ, Tamma PD, Milstone AM. 2016. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). Expert Rev Anti Infect Ther 14:95-108.
20. Gu D, Yan Z, Cai C, Li J, Zhang Y, Wu Y, Yang J, Huang Y, Zhang R, Wu Y. 2023. Comparison of the NG-Test Carba 5, Colloidal Gold Immunoassay (CGI) Test, and Xpert Carba-R for the Rapid Detection of Carbapenemases in Carbapenemase-Producing Organisms. Antibiotics (Basel) 12.
21. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB, Jr., Jenkins SG, Limbago BM, Das S. 2017. Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among Enterobacteriaceae. J Clin Microbiol 55:2321-2333.
22. Satlin MJ, Chen L, Gomez-Simmonds A, Marino J, Weston G, Bhowmick T, Seo SK, Sperber SJ, Kim AC, Eilertson B, Derti S, Jenkins SG, Levi MH, Weinstein MP, Tang YW, Hong T, Juretschko S, Hoffman KL, Walsh TJ, Westblade LF, Uhlemann AC, Kreiswirth BN. 2022. Impact of a Rapid Molecular Test for Klebsiella pneumoniae Carbapenemase and Ceftazidime-Avibactam Use on Outcomes After Bacteremia Caused by Carbapenem-Resistant Enterobacterales. Clin Infect Dis 75:2066-2075.
23. CLSI. 2024. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 12th edition. CLSI M07., 12th ed.
24. CLSI. 2025. Performance Standards for Antimicrobial Susceptibility Testing, 35th edition. CLSI M100., 35th ed.
25. Weinstein MP, Lewis JS, 2nd. 2020. The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background, Organization, Functions, and Processes. J Clin Microbiol 58.
26. Humphries RM, Hindler JA, Epson E, Horwich-Scholefield S, Miller LG, Mendez J, Martinez JB, Sinkowitz J, Sinkowtiz D, Hershey C, Marquez P, Bhaurla S, Moran M, Pandes L, Terashita D, McKinnell JA. 2018. Carbapenem-Resistant Enterobacteriaceae Detection Practices in California: What Are We Missing? Clin Infect Dis 66:1061-1067.
27. EUCAST. 2023. Breakpoint tables for interpretation of MICs and zone diameters, version 13.0, 13.0 ed.
28. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V, European Network on C. 2012. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect 18:432-8.
29. Adler A, Ben-Dalak M, Chmelnitsky I, Carmeli Y. 2015. Effect of Resistance Mechanisms on the Inoculum Effect of Carbapenem in Klebsiella pneumoniae Isolates with Borderline Carbapenem Resistance. Antimicrob Agents Chemother 59:5014-7.
30. Brook I. 1989. Inoculum effect. Rev Infect Dis 11:361-8.
31. Lenhard JR, Bulman ZP. 2019. Inoculum effect of beta-lactam antibiotics. J Antimicrob Chemother 74:2825-2843.
32. Gutierrez A, Jain S, Bhargava P, Hamblin M, Lobritz MA, Collins JJ. 2017. Understanding and Sensitizing Density-Dependent Persistence to Quinolone Antibiotics. Mol Cell 68:1147-1154 e3.
33. Diaz-Tang G, Meneses EM, Patel K, Mirkin S, Garcia-Dieguez L, Pajon C, Barraza I, Patel V, Ghali H, Tracey AP, Blanar CA, Lopatkin AJ, Smith RP. 2022. Growth productivity as a determinant of the inoculum effect for bactericidal antibiotics. Sci Adv 8:eadd0924.
34. Udekwu KI, Parrish N, Ankomah P, Baquero F, Levin BR. 2009. Functional relationship between bacterial cell density and the efficacy of antibiotics. J Antimicrob Chemother 63:745-57.
35. Scheler O, Makuch K, Debski PR, Horka M, Ruszczak A, Pacocha N, Sozanski K, Smolander OP, Postek W, Garstecki P. 2020. Droplet-based digital antibiotic susceptibility screen reveals single-cell clonal heteroresistance in an isogenic bacterial population. Sci Rep 10:3282.
36. Tan C, Smith RP, Srimani JK, Riccione KA, Prasada S, Kuehn M, You L. 2012. The inoculum effect and band-pass bacterial response to periodic antibiotic treatment. Mol Syst Biol 8:617.
37. Dugatkin LA, Perlin M, Lucas JS, Atlas R. 2005. Group-beneficial traits, frequency-dependent selection and genotypic diversity: an antibiotic resistance paradigm. Proc Biol Sci 272:79-83.
38. Geyrhofer L, Ruelens P, Farr AD, Pesce D, de Visser J, Brenner N. 2023. Minimal Surviving Inoculum in Collective Antibiotic Resistance. mBio 14:e0245622.
39. Golikova MV, Strukova EN, Alieva KN, Ageevets VA, Avdeeva AA, Sulian OS, Zinner SH. 2023. Meropenem MICs at Standard and High Inocula and Mutant Prevention Concentration Inter-Relations:

Comparative Study with Non-Carbapenemase-Producing and OXA-48-, KPC- and NDM-Producing Klebsiella pneumoniae. Antibiotics (Basel) 12.
40. Pathak A, Angst DC, Leon-Sampedro R, Hall AR. 2023. Antibiotic-degrading resistance changes bacterial community structure via species-specific responses. ISME J 17:1495-1503.
41. Vega NM, Gore J. 2014. Collective antibiotic resistance: mechanisms and implications. Curr Opin Microbiol 21:28-34.
42. Eng RH, Cherubin C, Smith SM, Buccini F. 1985. Inoculum effect of beta-lactam antibiotics on Enterobacteriaceae. Antimicrob Agents Chemother 28:601-6.
43. Dingle TC, Gamage D, Gomez-Villegas S, Hanson BM, Reyes J, Abbott A, Burnham CD, Dien Bard J, Fritz S, Miller WR, Westblade LF, Zimmer B, Arias CA, Butler-Wu S, Group CCHIA-HW. 2022. Prevalence and Characterization of the Cefazolin Inoculum Effect in North American Methicillin-Susceptible Staphylococcus aureus Isolates. J Clin Microbiol 60:e0249521.
44. Jean B, Crolle M, Pollani C, Le Guilloux A, Martin-Blondel G, Tattevin P, Le Bot A, Luque Paz D, Guerin F, Cattoir V, Armand-Lefevre L, Gueye S, Lescure FX, Duval X, Massip C, Delobel P. 2024. beta-Lactam Inoculum Effect in Methicillin-Susceptible Staphylococcus aureus Infective Endocarditis. JAMA Netw Open 7:e2451353.
45. Rincon S, Carvajal LP, Gomez-Villegas SI, Echeverri AM, Rios R, Dinh A, Pedroza C, Ordonez KM, Nannini E, Sun Z, Fowler VG, Murray BE, Miller WR, Palzkill T, Diaz L, Arias CA, Reyes J. 2021. A Test for the Rapid Detection of the Cefazolin Inoculum Effect in Methicillin-Susceptible Staphylococcus aureus. J Clin Microbiol 59.
46. Harada Y, Morinaga Y, Kaku N, Nakamura S, Uno N, Hasegawa H, Izumikawa K, Kohno S, Yanagihara K. 2014. In vitro and in vivo activities of piperacillin-tazobactam and meropenem at different inoculum sizes of ESBL-producing Klebsiella pneumoniae. Clin Microbiol Infect 20:O831-9.
47. Lee DG, Murakami Y, Andes DR, Craig WA. 2013. Inoculum effects of ceftobiprole, daptomycin, linezolid, and vancomycin with Staphylococcus aureus and Streptococcus pneumoniae at inocula of 10(5) and 10(7) CFU injected into opposite thighs of neutropenic mice. Antimicrob Agents Chemother 57:1434-41.
48. Smith KP, Kirby JE. 2018. The Inoculum Effect in the Era of Multidrug Resistance: Minor Differences in Inoculum Have Dramatic Effect on MIC Determination. Antimicrob Agents Chemother 62.
49. Lutgring JD, Machado MJ, Benahmed FH, Conville P, Shawar RM, Patel J, Brown AC. 2018. FDA-CDC Antimicrobial Resistance Isolate Bank: a Publicly Available Resource To Support Research, Development, and Regulatory Requirements. J Clin Microbiol 56.
50. Ambler RP. 1980. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci 289:321-31.
51. Sawa T, Kooguchi K, Moriyama K. 2020. Molecular diversity of extended-spectrum beta-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care 8:13.
52. Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 177:4121-30.
53. Kim JY, Jung HI, An YJ, Lee JH, Kim SJ, Jeong SH, Lee KJ, Suh PG, Lee HS, Lee SH, Cha SS. 2006. Structural basis for the extended substrate spectrum of CMY-10, a plasmid-encoded class C beta-lactamase. Mol Microbiol 60:907-16.
54. Castanheira M, Simner PJ, Bradford PA. 2021. Extended-spectrum beta-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 3:dlab092.
55. Martinez-Martinez L, Pascual A, Hernandez-Alles S, Alvarez-Diaz D, Suarez AI, Tran J, Benedi VJ, Jacoby GA. 1999. Roles of beta-lactamases and porins in activities of carbapenems and cephalosporins against Klebsiella pneumoniae. Antimicrob Agents Chemother 43:1669-73.
56. Wang Y, Wang S, Chen W, Song L, Zhang Y, Shen Z, Yu F, Li M, Ji Q. 2018. CRISPR-Cas9 and CRISPR-Assisted Cytidine Deaminase Enable Precise and Efficient Genome Editing in Klebsiella pneumoniae. Appl Environ Microbiol 84.
57. Bottery MJ, Pitchford JW, Friman VP. 2021. Ecology and evolution of antimicrobial resistance in bacterial communities. ISME J 15:939-948.
58. Lomovskaya O, Sun D, Rubio-Aparicio D, Nelson K, Tsivkovski R, Griffith DC, Dudley MN. 2017. Vaborbactam: Spectrum of Beta-Lactamase Inhibition and Impact of Resistance Mechanisms on Activity in Enterobacteriaceae. Antimicrob Agents Chemother 61.

59. Ma P, He LL, Pironti A, Laibinis HH, Ernst CM, Manson AL, Bhattacharyya RP, Earl AM, Livny J, Hung DT. 2021. Genetic determinants facilitating the evolution of resistance to carbapenem antibiotics. Elife 10.
60. Adelman MW, Bower CW, Grass JE, Ansari UA, Soda EA, See I, Lutgring JD, Jacob JT. 2022. Distinctive Features of Ertapenem-Mono-Resistant Carbapenem-Resistant Enterobacterales in the United States: A Cohort Study. Open Forum Infect Dis 9:ofab643.
61. Doumith M, Ellington MJ, Livermore DM, Woodford N. 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. J Antimicrob Chemother 63:659-67.
62. Tsai YK, Liou CH, Fung CP, Lin JC, Siu LK. 2013. Single or in combination antimicrobial resistance mechanisms of Klebsiella pneumoniae contribute to varied susceptibility to different carbapenems. PLoS One 8:e79640.
63. Sykes RB, Matthew M. 1976. The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. J Antimicrob Chemother 2:115-57.
64. Artemova T, Gerardin Y, Dudley C, Vega NM, Gore J. 2015. Isolated cell behavior drives the evolution of antibiotic resistance. Mol Syst Biol 11:822.
65. Meredith HR, Srimani JK, Lee AJ, Lopatkin AJ, You L. 2015. Collective antibiotic tolerance: mechanisms, dynamics and intervention. Nat Chem Biol 11:182-8.
66. Tanouchi Y, Pai A, Buchler NE, You L. 2012. Programming stress-induced altruistic death in engineered bacteria. Mol Syst Biol 8:626.
67. Gross R, Mungan M, Das SG, Yüksel M, Maier B, Bollenbach T, Krug J, de Visser JAGM. 2024. Collective beta-lactam resistance in Escherichia coli due to beta-lactamase release upon cell death. bioRxiv doi:https://doi.org/10.1101/2024.10.14.618215.
68. Kim SW, Park SB, Im SP, Lee JS, Jung JW, Gong TW, Lazarte JMS, Kim J, Seo JS, Kim JH, Song JW, Jung HS, Kim GJ, Lee YJ, Lim SK, Jung TS. 2018. Outer membrane vesicles from beta-lactam-resistant Escherichia coli enable the survival of beta-lactam-susceptible E. coli in the presence of beta-lactam antibiotics. Sci Rep 8:5402.
69. Martinez MMB, Bonomo RA, Vila AJ, Maffia PC, Gonzalez LJ. 2021. On the Offensive: the Role of Outer Membrane Vesicles in the Successful Dissemination of New Delhi Metallo-beta-lactamase (NDM-1). mBio 12:e0183621.
70. Tang B, Yang A, Liu P, Wang Z, Jian Z, Chen X, Yan Q, Liang X, Liu W. 2023. Outer Membrane Vesicles Transmitting bla(NDM-1) Mediate the Emergence of Carbapenem-Resistant Hypervirulent Klebsiella pneumoniae. Antimicrob Agents Chemother 67:e0144422.
71. Opota O, Croxatto A, Prod'hom G, Greub G. 2015. Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 21:313-22.
72. Zhang C, Cleveland K, Schnoll-Sussman F, McClure B, Bigg M, Thakkar P, Schultz N, Shah MA, Betel D. 2015. Identification of low abundance microbiome in clinical samples using whole genome sequencing. Genome Biol 16:265.
73. Miller WR, Seas C, Carvajal LP, Diaz L, Echeverri AM, Ferro C, Rios R, Porras P, Luna C, Gotuzzo E, Munita JM, Nannini E, Carcamo C, Reyes J, Arias CA. 2018. The Cefazolin Inoculum Effect Is Associated With Increased Mortality in Methicillin-Susceptible Staphylococcus aureus Bacteremia. Open Forum Infect Dis 5:ofy123.
74. Nannini EC, Stryjewski ME, Singh KV, Bourgogne A, Rude TH, Corey GR, Fowler VG, Jr., Murray BE. 2009. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible Staphylococcus aureus: frequency and possible cause of cefazolin treatment failure. Antimicrob Agents Chemother 53:3437-41.
75. Hovan MR, Narayanan N, Cedarbaum V, Bhowmick T, Kirn TJ. 2021. Comparing mortality in patients with carbapenemase-producing carbapenem resistant Enterobacterales and non-carbapenemase-producing carbapenem resistant Enterobacterales bacteremia. Diagn Microbiol Infect Dis 101:115505.
76. Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, Simner PJ. 2017. Comparing the Outcomes of Patients With Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae Bacteremia. Clin Infect Dis 64:257-264.
77. Simner PJ, Pitout JDD, Dingle TC. 2024. Laboratory detection of carbapenemases among Gram-negative organisms. Clin Microbiol Rev 37:e0005422.

78. Fouad A, Gill CM, Simner PJ, Nicolau DP, Asempa TE. 2023. Cefepime in vivo activity against carbapenem-resistant Enterobacterales that test as cefepime susceptible or susceptible-dose dependent in vitro: implications for clinical microbiology laboratory and clinicians. J Antimicrob Chemother 78:2242-2253.
79. Jean SS, Lee WS, Hsueh PR, Group SA-P. 2018. Ertapenem non-susceptibility and independent predictors of the carbapenemase production among the Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). Infect Drug Resist 11:1881-1891.
80. Lamoureaux TL, Frase H, Antunes NT, Vakulenko SB. 2012. Antibiotic resistance and substrate profiles of the class A carbapenemase KPC-6. Antimicrob Agents Chemother 56:6006-8.
81. Stewart NK, Smith CA, Frase H, Black DJ, Vakulenko SB. 2015. Kinetic and structural requirements for carbapenemase activity in GES-type beta-lactamases. Biochemistry 54:588-97.
82. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. 2012. Rapid detection of carbapenemase genes by multiplex real-time PCR. J Antimicrob Chemother 67:906-9.
83. Tamma PD, Simner PJ. 2018. Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. J Clin Microbiol 56.
84. Vazquez-Ucha JC, Arca-Suarez J, Bou G, Beceiro A. 2020. New Carbapenemase Inhibitors: Clearing the Way for the beta-Lactams. Int J Mol Sci 21.
85. Elawady B, Ghobashy M, Balbaa A. 2019. Rapidec Carba NP for Detection of Carbapenemase-Producing Enterobacteriaceae in Clinical Isolates: A Cross-Sectional Study. Surg Infect (Larchmt) 20:672-676.
86. MacLean RC, San Millan A. 2019. The evolution of antibiotic resistance. Science 365:1082-1083.
87. Babouee Flury B, Ellington MJ, Hopkins KL, Turton JF, Doumith M, Loy R, Staves P, Hinic V, Frei R, Woodford N. 2016. Association of Novel Nonsynonymous Single Nucleotide Polymorphisms in ampD with Cephalosporin Resistance and Phylogenetic Variations in ampC, ampR, ompF, and ompC in Enterobacter cloacae Isolates That Are Highly Resistant to Carbapenems. Antimicrob Agents Chemother 60:2383-90.
88. Foudraine DE, Strepis N, Stingl C, Ten Kate MT, Verbon A, Klaassen CHW, Goessens WHF, Luider TM, Dekker LJM. 2021. Exploring antimicrobial resistance to beta-lactams, aminoglycosides and fluoroquinolones in E. coli and K. pneumoniae using proteogenomics. Sci Rep 11:12472.
89. Pesesky MW, Hussain T, Wallace M, Patel S, Andleeb S, Burnham CD, Dantas G. 2016. Evaluation of Machine Learning and Rules-Based Approaches for Predicting Antimicrobial Resistance Profiles in Gram-negative Bacilli from Whole Genome Sequence Data. Front Microbiol 7:1887.
90. Shelburne SA, Kim J, Munita JM, Sahasrabhojane P, Shields RK, Press EG, Li X, Arias CA, Cantarel B, Jiang Y, Kim MS, Aitken SL, Greenberg DE. 2017. Whole-Genome Sequencing Accurately Identifies Resistance to Extended-Spectrum beta-Lactams for Major Gram-Negative Bacterial Pathogens. Clin Infect Dis 65:738-745.
91. Suelter CS, Hanson ND. 2020. OmpC regulation differs between ST131 and non-ST131 Escherichia coli clinical isolates and involves differential expression of the small RNA MicC. J Antimicrob Chemother 75:1151-1158.
92. Wiegand I, Hilpert K, Hancock RE. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3:163-75.