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The effect of intestinal microbiota dysbiosis on growth and detection of carbapenemase-producing Enterobacterales within an in vitro gut model

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Running title

Detection of CPE in an in vitro gut model

Summary

Background: Carbapenemase-producing Enterobacterales (CPE) can colonise the gut and are of major clinical concern. Identification of CPE colonisation is problematic; there is no gold-standard detection method, and the effects of antibiotic exposure and microbiota dysbiosis on detection are unknown.

Aim: Based on a national survey we selected four CPE screening assays in common use. We used a clinically-reflective *in vitro* model of human gut microbiota to investigate the performance of each test to detect three different CPE strains under different, clinically-relevant antibiotic exposures.

Methods: Twelve gut models were seeded with a pooled faecal slurry and exposed to CPE either before, after, concomitant with, or in the absence of piperacillin-tazobactam (358 mg/l, 3x daily, 7 days). Total Enterobacterales and CPE populations were enumerated daily. Regular screening for CPE was performed using Cepheid Xpert® Carba-R molecular test, and with *Brilliance™ CRE*, Colorex™ mSuperCARBA and CHROMID® CARBA SMART agars.

Findings: Detection of CPE when the microbiota are intact is problematic. Antibiotic exposure disrupts microbiota populations and allows CPE proliferation, increasing detection. The performances of assays varied, particularly with respect to different CPE strains. The Cepheid assay performed better than the three agar methods for detecting a low level of CPE within an intact microbiota, although performance of all screening methods was comparable when CPE populations increased in a disrupted microbiota.

Conclusion: CPE strains differed in their dynamics of colonisation in an *in vitro* gut model and in their subsequent response to antibiotic exposure. This affected detection by molecular and screening methods, which has implications for the sensitivity of CPE screening in healthcare settings.

Key words: Carbapenemase-producing Enterobacterales (CPE), gut microbiota, dysbiosis, screening, antibiotics.

Introduction

Antibiotic resistance in clinically relevant bacteria is of increasing concern. In particular, the prevalence of carbapenemase-producing Enterobacterales (CPE) has increased in recent years,

becoming an important threat to public health.[1-3] There are numerous carbapenemases, however, isolates containing the so-called 'big five' carbapenemase families (IMP, KPC, NDM, OXA-48-like and VIM) predominate among those submitted to the PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for investigation of carbapenem resistance.[4] These enzymes can render bacteria resistant to most beta-lactam antibiotics, reducing the number of treatment options.

Carbapenemases are increasingly identified in Enterobacterales that form part of the normal gut microbiota. Thus, CPE may colonise patients, as part of their (altered) gut microbiota, [5] and spread between asymptomatic individuals, particularly in healthcare settings.[6] A recent prospective European study demonstrated that 1.3 patients per 10,000 hospital admissions had CPE-positive clinical specimens, although this rate varied considerably between countries.[3]

Rapid, accurate detection of these organisms in patients is paramount to ensure appropriate patient management and infection control procedures are put in place to minimise spread.[7] However, this is complicated by a number of factors, not least determining which patients to screen for CPE carriage. It is unrealistic and expensive to test all patients in most hospitals (this is not considered to be cost-effective[7]), so detection is usually targeted towards 'at risk' patient subgroups, defined according to local epidemiology and prevalence.[7, 8]. Importantly, very little is known about the impact of antibiotic therapy on the detection of CPE, and this factor is rarely considered when screening patients.

There is no 'gold standard' method for detection of CPE in stool samples or rectal swabs; UK Standards for Microbiology Investigations (SMI) guidelines recommend using commercial selective chromogenic agar, [9] and many options for CPE detection are available. [10-15] Indeed, a recent survey found considerable heterogeneity in the screening methods used across England, and also in identification of patients targeted for screening.[16]

We investigated the growth and detection of three carbapenemase-producing *Klebsiella pneumoniae* strains in an *in vitro* model of the human colon in the presence of both a normal, healthy microbiota, and a dysbiotic microbiota disrupted by antibiotic exposure. We have previously demonstrated that this model can be used to investigate CPE intestinal colonisation.[17] This gut model has been used extensively to investigate the interplay between the intestinal microbiota, antibiotic exposure and *Clostridioides difficile* [18-20] and has been shown to be reproducible and clinically reflective. [21]

Methods

The *in vitro* gut model consists of a triple-stage chemostat system, water-jacketed at 37°C, top-fed with complex growth media and maintained at gut-reflective pH (pH 5.5 (±0.2), 6.2 (±0.2), 6.8 (±0.2) for vessel 1, 2 and 3, respectively). Vessel contents are magnetically stirred and sparged with nitrogen to maintain an anaerobic environment. Intestinal microbiota populations are established via a pooled slurry of faecal samples from healthy volunteers (n=5). Any healthy adult (age >18 years) with no history of antibiotic therapy in the last three months was eligible. Samples were anonymous and no participant information was collected. Samples were confirmed negative for CPE (by Cepheid Xpert® Carba-R assay and plating on at least one commercial agar specific for CPE detection). Each vessel has stoppered ports to facilitate interventions and sampling.

Experimental design

Twelve gut model experiments were run, evaluating the detection of three different CPE strains (outlined in Table 1) with and without prior exposure to antibiotics (Figure 1). Initial microbiota populations were established from faecal slurry and allowed to reach a steady state (~2 weeks) prior to any intervention. Initially, three models were run for each CPE strain; one was not exposed to

97 antimicrobials (models A, D, G), and the other two were instilled with a clinically reflective
98 antimicrobial dosing regimen (piperacillin/tazobactam - 358 mg/l, 3x daily, 7 days), either before
99 (model B, E, H) or during (model C, F, I) CPE exposure (Figure 1). Faecal levels of piperacillin-
100 tazobactam have been shown to vary considerably [22, 23], so the dosing regimen has been chosen to
101 reflect reported biliary concentrations [24] as with previous gut model experiments [25]. These nine
102 models were exposed to daily increasing inocula of CPE strains (between 3 and 6 $\log_{10}$ cfu/mL diluted
103 from overnight cultures in nutrient broth) over a 5-8 day period. A further three models (J,K,L) were
104 run in which two, single inocula of CPE (1 mL of a 1:10,000 dilution of the overnight culture) were
105 added into an intact microbiota, one week apart, followed by instillation of the same
106 piperacillin/tazobactam regimen (Figure 1). All models were sampled daily and total
107 Enterobacterales populations (MacConkey agar), and CPE populations (BioMerieux CHROMID®
108 CARBA SMART) enumerated. Other microbiota populations were also regularly enumerated on
109 selective and non-selective agars (appendix 1). Four different detection assays (selected to reflect
110 current practices in the UK)[16] were used to detect CPE at each of the screening times outlined in
111 Figure 1.

112 *Screening methods*

113 Screening methods used for CPE detection were;

114 • Cepheid Xpert® Carba-R assay molecular assay (Cepheid)
115 • *Brilliance™ CRE* agar (Oxoid)
116 • Colorex™ mSuperCARBA™ agar (E&O Laboratories)
117 • CHROMID® CARBA SMART agar (bioMérieux)

118 For all screening methods, collection swabs (Cepheid) were submerged in aliquots (~3mL) of gut
119 model fluid taken from vessel 3. Each aliquot was tested in triplicate at each time point, i.e. three
120 swabs were submerged in the same aliquot of fluid, prior to being used according to manufacturer's
121 instructions for processing a clinical rectal swab.

CPE strains

We evaluated the detection of three different CPE strains within the gut model (Table 1). Strains were isolated from clinical samples of patients at Leeds Teaching Hospitals Trust (LTHT) and were selected as producers of three prevalent carbapenemases; KPC, NDM and the often difficult to detect OXA-48.[26]

Results

Growth dynamics of CPE in the gut model

CPE populations were enumerated regularly to allow the growth dynamics of the CPE within the gut microbiota environment to be investigated.

The instillation of piperacillin-tazobactam into gut models caused marked disruption to the microbiota. All measured microbiota populations were affected, most notably bifidobacteria, *Bacteroides fragilis* group and lactobacilli, often reduced below the limit of detection (appendix 2). In models that received no antibiotics (A, D, G), microbiota disruption was not observed, with the exception of a reduction in bifidobacteria in model D.

CPE populations remained low when inoculated into an intact microbiota. In all cases, piperacillin-tazobactam exposure caused an initial decline in Enterobacterales (Figure 2), which soon recovered, driven by an expansion of CPE. However, differences in the growth dynamics of the three different strains were observed.

K. pneumoniae OXA-48 (KP-OXA-48): Models A (no antibiotics), B (prior antibiotics), C (concomitant antibiotics), J (post antibiotics)

Increasing inocula of KP-OXA-48 were added to models A, B and C from day 28 (3.26 log_{10} cfu) up to day 35 (5.12 log_{10} cfu). Due to lack of CPE detection, an additional inoculum of 6.25 log_{10} cfu was added into model A on day 39. For model J, two aliquots of 2.7 log_{10} cfu were added on days 14 and 21 (Figure 3).

KP-OXA-48 populations did not establish in an intact microbiota in the absence of antibiotics (Figures 2A, 3J), even following the largest inoculum used (6.25 log_{10} cfu). However, when introduced into a disrupted microbiota (model B), KP-OXA-48 was detectable following the lowest inoculum (3.26 log_{10} cfu). In all models, CPE populations quickly proliferated following antibiotic exposure to ~8 log_{10} cfu/mL, equal to total Enterobacterales indicating that immediately post antibiotic exposure, the majority of Enterobacterales were carbapenemase producers (Figures 2B, 2C, 2J).

K. pneumoniae NDM (KP-NDM): Models D (no antibiotics), E (prior antibiotics), F (concomitant antibiotics), K (post antibiotics)

Increasing inocula of KP-NDM were added to models D, E and F from day 23 (3.49 log_{10} cfu) up to day 30 (5.62 log_{10} cfu). For model K, two aliquots of 2.1 log_{10} cfu were added on days 14 and 21. (Figure 3)

Total Enterobacterales populations remained fairly stable in the absence of antibiotic exposure, and CPE populations did not establish until after the highest inoculum of KP-NDM in model D (Figure 2D), or the second inoculum KP-NDM in model K (Figure 2K). In model D, CPE remained at around the limit of detection, whereas in model K, CPE increased to ~4log_{10} cfu/mL. In models E and F, following piperacillin-tazobactam exposure, a non-CPE population was detected on Colorex™ mSuperCARBA agar plates (prior to KP-NDM inoculation in model E). These colonies did not contain an NDM gene (as confirmed by PCR) and did not grow on CHROMID® CARBA SMART agar, and are likely to represent a separate organism present in the initial faecal slurry proliferating after antibiotic

exposure. Enumeration of CPE on both CHROMID® CARBA SMART agar and Colorex™ mSuperCARBA agar is therefore presented for these models (Figures 2E, 2F).

When inoculated into a disrupted microbiota (model E) KP-NDM (confirmed by MALDI-TOF and PCR) was detected following the lowest inoculum (3.49 $\log_{10}$ cfu) and populations quickly proliferated to ~6 $\log_{10}$ cfu (Figure 2E). When piperacillin-tazobactam and *K. pneumoniae* NDM were inoculated concomitantly into model F, KP-NDM was detected on day 28 following an inoculation of 4.54 $\log_{10}$ cfu, and increased, but to a much lower level than in model E (~3 $\log_{10}$ cfu/mL – Figure 2F). In model K, instillation of piperacillin-tazobactam caused a rapid decline in CPE populations initially (day 30), but these quickly recovered and increased to >8 $\log_{10}$ cfu/mL by the end of the antibiotic dosing period. Interestingly, molecular testing revealed the emergence of an additional CPE population in this model (model K). Prior to antibiotic exposure, only the NDM gene was detected, but from day 34, the KPC gene also began to be detected, indicating that from this point onwards, the enumerated CPE populations comprised both NDM and KPC encoding organisms.

K. pneumoniae KPC (KP-KPC): Models G (no antibiotics), H (prior antibiotics), I (concomitant antibiotics), L (post antibiotics)

Increasing inocula of KP-KPC were added to models G, H and I from day 26 (3.53 $\log_{10}$ cfu) up to day 30 (4.43 $\log_{10}$ cfu). For model L, two aliquots of 3.3 $\log_{10}$ cfu were added on days 14 and 21 (Figure 3).

In the absence of antibiotic mediated microbiota disruption, KP-KPC was detectable following an inoculum of 4.03 $\log_{10}$ cfu in model G (Figure 2G), and following the second KP-KPC inoculum in model L. In both these models CPE populations increased to ~6 $\log_{10}$ cfu/mL despite the absence of antibiotic exposure. In all models inoculated with KP-KPC, CPE population growth was rapid (Figures

2G, 2H, 2I, 2L), but particularly in those where the microbiota was disrupted with antibiotics (Figures 2H, 2I, 2L).

Notably, although the CPE strains inoculated into the model were all *K. pneumoniae*, other resistant Enterobacterales, particularly *E. coli* were isolated on both enumeration and screening plates. This was particularly evident in models inoculated with the KP-KPC strain.

Detection of CPE using the different screening methods

Swabs submerged in gut model fluid (to mimic a rectal swab) were regularly collected in order to compare the four different screening platforms at different points in the experiment.

Effect of CPE isolate (carbapenemase gene) on detection

Differences were observed between the performance of the detection methods for different isolates.

The molecular test (Cepheid) had a lower limit of detection for KP-OXA-48 than other methods and was the only screening test to detect it in a diverse microbiota (models A and J, Figure 3). Of the agars tested, Brilliance™ CRE was the most consistent in detection of KP-OXA-48. Interestingly, the OXA side of the CHROMID® CARBA SMART bi-plate, which is designed to help identify hard-to-detect OXA-48 – like containing organisms, was the least consistent in detection of this KP-OXA-48 strain, and had a much higher limit of detection for this strain than the CARBA side.

For the KP-NDM strain, it appeared that the Colorex™ mSuperCARBA agar had the lowest limit of detection, identifying this CPE strain earlier in the experiment than any other method. However, further molecular analysis on growth from Colorex™ mSuperCARBA plates showed no NDM gene was present (indeed, no carbapenemase gene was detected), suggesting this agar may be prone to false positives. The Cepheid molecular test therefore appeared to have the lowest limit of detection

for KP-NDM, identifying it more frequently (particularly in model F) than the remaining agar tests (Brilliance™ CRE and CHROMID® CARBA SMART).

Three screening methods had a similar limit of detection for KP-KPC; the Cepheid molecular platform, Colorex™ mSuperCARBA agar and CHROMID® CARBA SMART agar. Both the Cepheid Xpert® and Colorex™ mSuperCARBA platforms had sporadic positive screens (in models G and I respectively), but generally, these three methods detected CPE at the same point in the experiments. However, the Brilliance™ CRE agar performed substantially worse at detecting this CPE strain, consistently not returning a positive result until later than other tests (6, 1 and 2 days later in models G, H and I respectively). Interestingly, the Brilliance™ CRE agar was also negative for CPE at the end of the experiment in models H, I, and especially L, despite enumerated CPE remaining high, and other screening platforms detecting CPE at this point.

Effect of Host Microbiota on detection

The state of the host microbiota had a marked effect on the behaviour of CPE within the gut model, which in turn markedly impacted on CPE detection. When CPE were inoculated into an intact microbiota (models A, D, G, J, K, L), a much larger inoculum was required before screening platforms could detect the resistant organisms. Crucially, in models A and J (inoculated with KP-OXA-48) and D (inoculated with KP-NDM) only sporadic positive screens were observed despite inoculation of >5 $\log_{10}$ cfu/mL. Only in models inoculated with KP-KPC (G and L) was CPE consistently detected in an intact microbiota.

Conversely, when CPE were inoculated into a disrupted microbiota (models B, E and H), they were rapidly detected following the lowest inoculum. Due to the rapid proliferation of CPE strains in a disrupted microbiota, the majority of screening methods evaluated were consistently positive for CPE detection and appeared to have comparable performance and limits of detection, although some strain-specific variation was observed as discussed above.

Effect of Antibiotic Exposure on detection

Enumeration demonstrated that when CPE were inoculated into an intact microbiota concomitant to antibiotic exposure (models C, F, and I), proliferation of CPE occurred as other microbiota species were disrupted. This was reflected in the performance and limit of detection of the screening platforms. Interestingly in model J (inoculated with KP-OXA-48), CPE were detected only sporadically following inoculation, however by 5 days post antibiotic exposure, CPE was detected in all models by all screening platforms (Figure 3).

Discussion

Using an *in vitro* gut model system, we have simulated CPE exposure and colonisation of the gut and demonstrated that the status of the microbiota and antibiotic exposure affects CPE population dynamics and detection in a strain-dependant manner. This model system has been validated using chemical and microbiological measurements on the intestinal content of sudden death victims, [27] and has been shown to model the interplay between the microbiota, antibiotics and *C. difficile* infection in a clinically reflective way. [21] We have previously demonstrated that this model can be used to investigate interplay between the microbiota and CPE, [17] although how this reflects clinical CPE behaviour is not yet clear.

We demonstrated that when the intestinal microbiota is intact, large and/ or repeated CPE exposure can be required for CPE populations to proliferate and establish in large numbers. This was particularly evident for the KP-OXA-48 strain and is in line with previously described 'colonisation resistance' to CPE, which has led to the investigation of Faecal Microbiota Transplantation (FMT) as a 'decolonisation' method.[28-30] It is important to note, however, that 'decolonisation' more likely equates to a reduction in population density to below the limit of detection. Indeed, our data suggest that CPE can be present in an intact microbiota below the limit

of detection for a matter of weeks, but can subsequently proliferate and become detectable, e.g. following antibiotic exposure. This could have major implications for the way FMT donor stools are screened for CPE and other MDRO, particularly given the recent safety alert issued by the FDA following deaths associated with MDRO transmission events.[31] In these cases, organisms were not detected due to a lack of screening (and subsequent screening of stored stool identified ESBL-producing E-coli identical to that in the patients), however, our data indicates that for the CPE strains investigated here, a lack of detection in healthy stool samples may not be sufficient to ensure no CPE are present, and hence transferred to vulnerable recipients.

The KP-KPC strain established most readily in the intact microbiota, tended to proliferate most readily and was associated with rapid dissemination of resistance genes into other Enterobacterales populations, confirming both previous observations in the gut model [17], and the described wide dissemination and plasticity of these genes and associated mobile genetic elements.[32] The emergence of an unexpected KPC encoding population in model K following antibiotic exposure also points to increased dissemination and plasticity of these genes. It is not clear whether the emergence of this KPC containing population is due to the accidental introduction of a KPC containing organism, or whether it was present in the original stool below the limit of detection.

We have concentrated on investigating the performance of one molecular and three agar screening methods to reflect those used most commonly across England.[16] Importantly, we found that the performance of the screening methods varied according to the CPE strain, and was also affected by the composition of the microbiota populations within the gut environment. The Cepheid molecular platform proved to be the most consistent test across all conditions. When microbiota populations were disrupted, and CPE proliferation was high, performance of the CHROMID® CARBA SMART agar was similar to that of the Cepheid, however, in an intact microbiota, where levels of CPE remained low, the Cepheid platform detected CPE more frequently. Performance of agar methods were variable and particularly influenced by CPE strain (e.g. the high limit of detection of the Brilliance™

289 CRE agar for the KP-KPC strain used here), and specificity issues (false positive detection by the
290 Colorex™ mSuperCARBA agar of a population of non-CPE encoding *K. pneumonia* was noted). Of
291 the agars, the CHROMID® CARBA SMART was the most consistent in CPE detection, although it was
292 interesting to note that the 'OXA-48' portion of this biplate did not reliably detect this KP-OXA-48
293 strain, whereas the 'CARBA' portion did.

294 It is important to note that only three strains representative (in terms of carbapenemase gene) of
295 those in patients in Leeds Teaching Hospitals Trust were investigated in this study. Given the
296 marked strain-to-strain differences observed, use of different CPE strains may have led to different
297 relative test performances. The use of different CPE strains should therefore be carefully considered
298 when comparing detection platforms.

299 Multiple comparisons of various agars for the detection of CPE have been carried out, and huge
300 variation has been reported in performance [15, 33-35]. Many studies have indicated that performance
301 of screening tests is dependent on strain, and the background epidemiology of CPE will affect the
302 performance of detection assays. Particularly problematic strains such as OXA-244 producers have
303 been described, and the CHROMID® CARBA SMART agar has been shown to be less effective in
304 detection of these strains.[36] We found the Brilliance™ CRE agar to be particularly inconsistent for the
305 detection of *K. pneumoniae* KPC.

306 Taken together, these data show that the status of the host microbiota greatly affects the
307 population dynamics of CPE within the gut environment. This may have particular relevance in
308 healthcare settings, a known risk for CPE dissemination [6], and where patients are likely to receive
309 antibiotic treatments. Our data suggest that in certain cases patients exposed to CPE could screen
310 negative for the organisms, but that subsequent antibiotic exposure could lead to proliferation of a
311 previously undetected population within the gut environment, leading to these patients becoming
312 an infection control risk. Potentially the inclusion of antibiotic exposure as a new testing criterion in
313 high-risk patients should be considered, as outlined in the recently updated PHE framework. [7]

Importantly, in our experiments this was most likely to be the case for the KP-OXA-48 strain. OXA-48-like genes are hard to detect.[26] Molecular testing (as represented by the Cepheid here) has the advantage of identifying the CPE gene(s) present in a patient's gut flora, which can be crucial in outbreak investigation, reducing the need for confirmatory testing. It also has the advantage of speed, with a result returned ~1 hour after processing as compared with ~24 hours for agar methods. However, it should also be noted that isolation of the organism (achieved by agar but not molecular methods) can be desirable for longitudinal monitoring, epidemiological studies and determination of antibiotic susceptibilities. This is important in the detection of horizontal gene transfer and multi-species outbreaks. Crucially, it may be of more importance for screening laboratories to understand the underlying population of CPE strains in the healthcare settings they serve and take this into consideration when detecting their preferred screening assay. However, there is likely to be a benefit to regular use of different /multiple screening assays to ensure emergence of 'new' CPE populations are not missed.

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442 *Table I – CPE isolates to be investigated in the gut model. MIC = minimum inhibitory concentration;*

443 *ERT = ertapenem; IMI = imipenem; MER = meropenem*

Organism	**Carbapenemase gene**	**Plasmid** [17]	**Clinical details**	**MIC ERT**	**MIC IMI**	**MIC MER**
Klebsiella pneumoniae ST661	KPC	pKpQiL-D2	Rectal swab Female Age 73yrs	4 mg/L	8 mg/L	4 mg/L
Klebsiella pneumoniae	OXA-48	No information available	Rectal swab Male Age 55yrs	>32 mg/L	Not available	16 mg/L
Klebsiella pneumonia single-locus variant of ST147	NDM	>170 kb IncFIB/IncFII (previously undescribed)	Rectal swab Female Age 68yrs	>32 mg/L	1 mg/L	8mg/L

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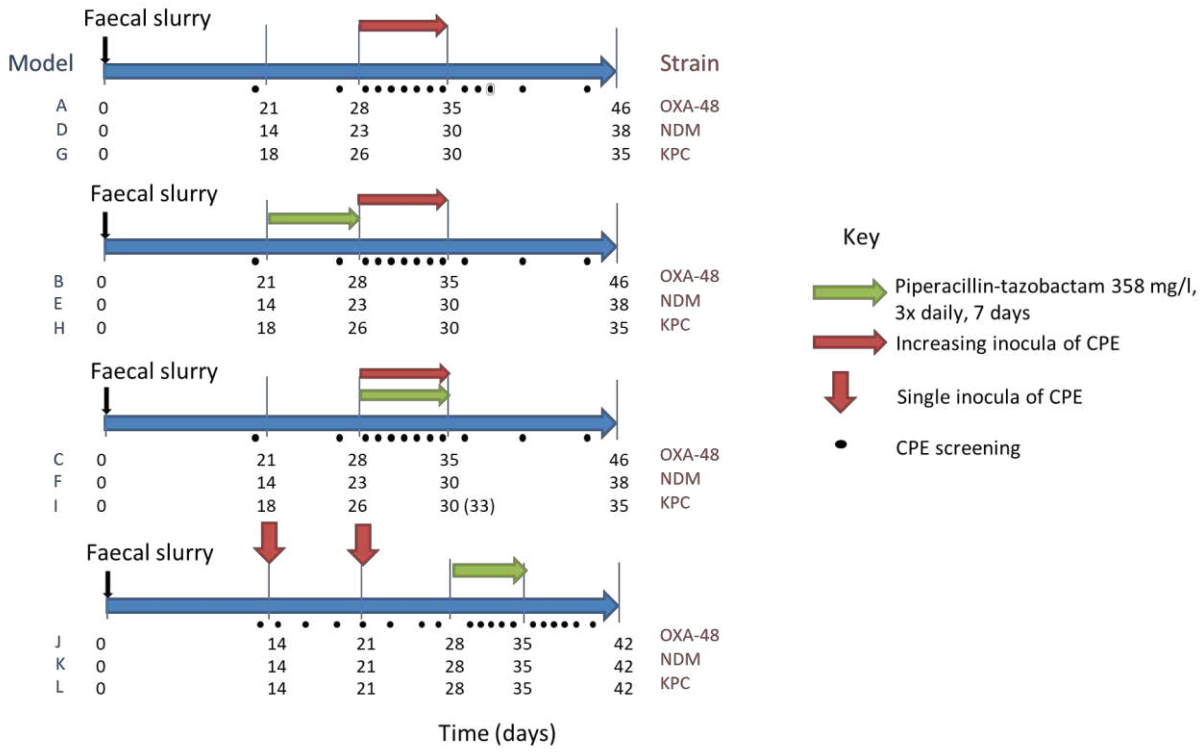

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Figure 1 – Gut model Experimental design. Timelines (in days) for each model is described. Green arrows represent antibiotic dosing periods. Red arrows represent CPE inoculation (either single aliquot or repeat increasing inocula). Black dots represent a CPE screening sampling timepoint. NB In model I the CPE was inoculated over a 4 day period (day 26-30) whereas the antibiotics were instilled over a 7 day period (ay 26-33).

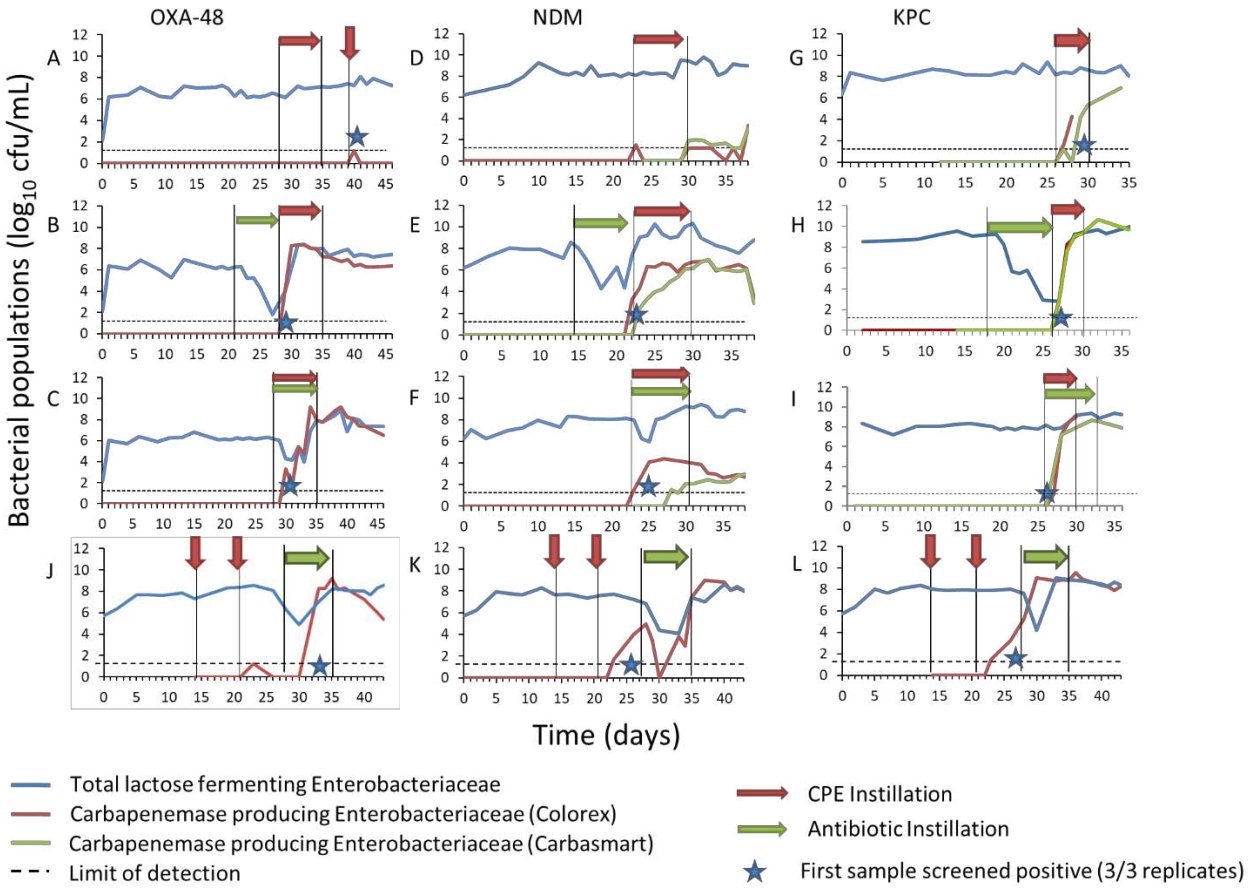

Figure 2 – Enterobacterales populations (log_{10} cfu/mL) in models A-L. Total Enterobacterales enumerated on MaConkey agar. Carbapenemase-producing Enterobacterales enumerated on Colorex™ mSuperCARBA and (in some models) CHROMID® CARBA SMART agars. Green arrows represent antibiotic dosing periods. Red arrows represent CPE inoculation (either single aliquot or repeat increasing inocula). Black dotted lines indicate limit of detection (1.22log_{10} cfu/mL). Blue

460 stars indicate the first sample in which all three aliquots of any screening assay were positive for

461 CPE.

OXA-48

	Day	CPE to vessel	Assay - no. replicates positive				
			Ceph	Colo	Brill	CS-Carb	CS - OXA
A	14		0/3	0/3	0/3	0/3	0/3
	21		0/3	0/3	0/3	0/3	0/3
	28	3.26	0/3	0/3	0/3	0/3	0/3
	29	3.49	0/3	0/3	0/3	0/3	0/3
	30	3.73	0/3	0/3	0/3	0/3	0/3
	31	3.94	1/3	0/3	0/3	0/3	0/3
	32	4.15	0/3	0/3	0/3	0/3	0/3
	33	4.15	0/3	0/3	0/3	0/3	0/3
	34	5.11	0/3	0/3	0/3	0/3	0/3
	35	5.12	0/3	0/3	0/3	0/3	0/3
	36		0/3	0/3	0/3	0/3	0/3
	39	6.25	0/3	0/3	0/3	0/3	0/3
	40		3/3	0/3	0/3	0/3	0/3
	41		1/3	0/3	0/3	0/3	0/3
	42		1/3	0/3	0/3	0/3	0/3
	43		1/3	0/3		0/3	0/3
	46		0/3	0/3		0/3	0/3
B	14		0/3	0/3	0/3	0/3	0/3
	21		0/3	0/3	0/3	0/3	0/3
	28	3.26	0/3	0/3	0/3	0/3	0/3
	29	3.49	3/3	3/3	3/3	2/3	0/3
	30	3.73	3/3	3/3	3/3	3/3	3/3
	31	3.94	3/3	3/3	3/3	3/3	3/3
	32	4.15	3/3	3/3	3/3	3/3	3/3
	33	4.15	3/3	3/3	3/3	3/3	3/3
	34	5.11	3/3	1/1	1/1	1/1	1/1
	35	5.12	3/3	2/2	1/1	1/1	1/1
	36		3/3	2/2	1/1	1/1	1/1
	39		3/3	1/1	1/1	1/1	0/1
	46		3/3	3/3		3/3	3/3
C	14		0/3	0/3	0/3	0/3	0/3
	21		0/3	0/3	0/3	0/3	0/3
	28	3.26	0/3	0/3	0/3	0/3	0/3
	29	3.49	0/3	0/3	0/3	0/3	0/3
	30	3.73	0/3	0/3	0/3	0/3	0/3
	31	3.94	3/3	1/3	3/3	1/3	0/3
	32	4.15	3/3	3/3	3/3	3/3	0/3
	33	4.15	3/3	3/3	3/3	3/3	0/3
	34	5.11	3/3	3/3	1/1	3/3	3/3
	35	5.12	3/3	3/3	1/1	1/1	1/1
	36		3/3	1/1	1/1	1/1	1/1
	39		3/3	1/1	1/1	1/1	1/1
	46		3/3	3/3		3/3	3/3
J	14	2.70	0/3	0/3	0/3	0/3	0/3
	16		0/3	0/3	0/3	0/3	0/3
	19		0/3	0/3	0/3	0/3	0/3
	21	2.70	0/3	0/3	0/3	0/3	0/3
	23		0/3	0/3	0/3	0/3	0/3
	26		0/3	0/3	0/3	0/3	0/3
	28		0/3	0/3	0/3	0/3	0/3
	29		0/3	0/3	0/3	0/3	0/3
	30		0/3	0/3	0/3	0/3	0/3
	33		3/3	3/3	3/3	3/3	3/3
	34		3/3	3/3	3/3	3/3	3/3
	35		3/3	3/3		3/3	3/3
	36		3/3	3/3	3/3	3/3	3/3
	37		3/3	3/3	2/2	3/3	3/3
	40		3/3	3/3	3/3	3/3	3/3
	41		3/3	3/3	3/3	3/3	3/3
	42		3/3	3/3	3/3	3/3	3/3
	43		3/3	3/3	3/3	3/3	3/3
	44		3/3	3/3	3/3	3/3	3/3

NDM

	Day	CPE to vessel	Assay - no. replicates positive				
			Ceph	Colo	Brill	CS-Carb	CS - OXA
D	15		0/3	0/3	0/3	0/3	0/3
	22		0/3	0/3	0/3	0/3	0/3
	23	3.49	0/3	0/3	0/3	0/3	0/3
	24	3.67	0/3	0/3	0/3	0/3	0/3
	25	4.06	0/3	0/3	0/3	0/3	0/3
	26	4.32	0/3	0/3	0/3	0/3	0/3
	27	4.54	0/3	0/3	0/3	0/3	0/3
	28	4.51	0/3	0/3	0/3	0/3	0/3
	29	5.02	1/3	0/3	0/3	0/3	0/3
	30	5.62	2/3	1/3	0/3	2/3	0/3
	31		0/3	0/3	0/3	1/3	0/3
	34		0/3	0/3	0/3	0/3	0/3
	40		0/3	0/1	0/1	0/3	0/3
E	15		0/3	0/3	0/3	0/3	0/3
	22		0/3	3/3	0/3	0/3	0/3
	23	3.49	0/3	3/3	1/3	0/3	1/3
	24	3.67	3/3	3/3	3/3	3/3	0/3
	25	4.06	3/3	3/3	3/3	3/3	0/3
	26	4.32	3/3	3/3	3/3	3/3	0/3
	27	4.54	3/3	3/3	3/3	3/3	0/3
	28	4.51	3/3	3/3	3/3	3/3	0/3
	29	5.02	3/3	3/3	2/2	2/2	0/2
	30	5.62	3/3	3/3	2/2	3/3	0/3
	31		3/3	3/3	1/1	3/3	0/3
	34		3/3	3/3	3/3	3/3	0/3
	40		3/3	1/1	1/1	3/3	0/3
F	15		0/3	0/3	0/3	0/3	0/3
	22		0/3	0/3	0/3	0/3	0/3
	23	3.49	0/3	1/3	0/3	0/3	0/3
	24	3.67	0/3	0/3	0/3	0/3	0/3
	25	4.06	0/3	3/3	0/3	0/3	0/3
	26	4.32	0/3	3/3	0/3	0/3	0/3
	27	4.54	0/3	3/3	0/3	0/3	0/3
	28	4.51	1/3	3/3	0/3	0/3	0/3
	29	5.02	2/3	3/3	0/3	0/3	0/3
	30	5.62	3/3	3/3	0/3	0/3	0/3
	31		1/3	3/3	0/3	0/3	0/3
	34		1/3	3/3	1/3	0/3	0/3
	40		3/3	1/1	1/1	3/3	0/3
K	14	3.3	0/3	0/3	0/3	0/3	0/3
	16		0/3	0/3	0/3	0/3	0/3
	19		0/3	0/3	0/3	0/3	0/3
	21	3.3	1/3	0/3	0/3	1/3	0/3
	23		0/3	0/3	1/3	0/3	0/3
	26		3/3	3/3	3/3	3/3	0/3
	28		3/3	3/3	3/3	3/3	0/3
	29		3/3	3/3	3/3	3/3	0/3
	30		3/3	2/3	3/3	2/3	0/3
	33		3/3	1/3	0/3	0/3	0/3
	34		3/3	3/3	3/3	3/3	0/3
	35		3/3	3/3	3/3	3/3	0/3
	36		3/3	3/3	3/3	3/3	3/3
	37		3/3	3/3	3/3	3/3	0/3
	40		3/3	3/3	3/3	3/3	0/3
	41		3/3	3/3	3/3	3/3	0/3
	42		3/3	3/3	3/3	3/3	0/3
	43		3/3	3/3	3/3	3/3	0/3
	44		3/3	3/3	3/3	3/3	0/3

KPC

	Day	CPE to vessel	Assay - no. replicates positive				
			Ceph	Colo	Brill	CS-Carb	CS - OXA
G	19		0/3	0/3	0/3	0/3	0/3
	26	3.53	0/3	0/3	0/3	0/3	0/3
	27	3.85	0/3	0/3	0/3	0/3	0/3
	28	4.03	0/3	0/3	0/3	0/3	0/3
	29	4.24	1/3	0/3	0/3	0/3	0/3
	30	4.43	3/3	1/1	0/3	3/3	0/3
	31		3/3	1/1	0/2	3/3	0/3
	34		3/3	1/1	0/2	3/3	0/3
	36		3/3		3/3	3/3	0/3
H	19		0/3	0/3	0/3	0/3	0/3
	26	3.53	0/3	0/3	0/3	0/3	0/3
	27	3.85	3/3	2/3	0/3	3/3	0/3
	28	4.03	3/3	3/3	3/3	3/3	0/3
	29	4.24	3/3	3/3	3/3	3/3	0/3
	30	4.43	3/3	1/1	3/3	3/3	0/3
	31		3/3	1/1	1/1	3/3	0/3
	34		3/3	1/1	3/3	3/3	0/3
	36		3/3	1/1	0/3	3/3	0/3
I	19		0/3	0/3	0/3	0/3	0/3
	26	3.53	0/3		0/3	0/3	0/3
	27	3.85	0/3	1/1	0/3	0/3	0/3
	28	4.03	3/3	3/3	0/3	3/3	0/3
	29	4.24	3/3	3/3	1/3	3/3	0/3
	30	4.43	3/3	1/1	3/3	3/3	0/3
	31		3/3	1/1	1/1	3/3	0/3
	34		3/3		3/3	3/3	0/3
	36		3/3	1/1	1/3	3/3	0/3
L	14	2.1	0/3	0/3	0/3	0/3	0/3
	16		0/3	0/3	0/1	0/3	0/3
	19		0/3	0/3	0/3	0/3	0/3
	21	2.1	0/3	0/3	0/3	0/3	0/3
	23		0/3	0/3	0/3	0/3	0/3
	26		2/3	3/3	0/3	3/3	0/3
	28		3/3	3/3	0/3	3/3	0/3
	29		3/3	3/3	3/3	3/3	0/3
	30		3/3	3/3	3/3	3/3	0/3
	33		3/3	3/3	3/3	3/3	0/3
	34		3/3	3/3	3/3	3/3	0/3
	35		3/3	3/3		3/3	0/3
	36		3/3	3/3	3/3	3/3	0/3
	37		3/3	3/3	0/2	3/3	0/3
	40		3/3	3/3	0/3	3/3	0/3
	41		3/3	3/3	0/3	3/3	0/3
	42		3/3	3/3	0/3	3/3	0/3
	43		3/3	3/3	0/3	3/3	0/3
	44		3/3	3/3	0/3	3/3	0/3

	Oxa-48	NDM	KPC
No antibiotics	A	D	G
Prior antibiotics	B	E	H
Concominant antibiotics	C	F	I
Post antibiotics	J	K	L

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463

464 Figure 3 – Results of screening assays in all gut model experiments. Ceph = Cepheid Gene Xpert®

465 molecular assay [Cepheid]; Colo = Colorex™ mSuperCARBA agar [E&O Laboratories; Brill =

466 Brilliance™ CRE CPE agar [Oxoid]; CS-Carb = CHROMID® CARBA SMART biplate agar [bioMérieux]

Carb side; CS-OXA = CHROMID® CARBA SMART biplate agar [bioMérieux] OXA side. Red bars indicate the inoculation of CPE (log_{10} cfu) into vessel 1 on that day (pre screening). Blue cells indicate negative results for all replicates (maximum 3). Dark yellow cells indicate positive results for all replicates. Light yellow cells indicate some replicates, but not all replicates were positive for CPE. The table (bottom R) indicates the timing of antibiotic exposure relative to CPE exposure.