Supplementary Material

Supplementary Methods

Population monitoring

Bacterial populations were sampled from (the distal) vessel 3 (V3) of the gut model. During sampling, an aliquot of gut model fluid was diluted in a 10-fold series dilution up to 10-7 in peptone water and used to enumerate both the indigenous gut populations and the resistance CPE populations. For indigenous gut populations, plates were inoculated in triplicate using 20 µL of the appropriate dilution series (up to 10-7) and incubated at 37°C.

DNA Extraction and Quality Control

Selected isolated were sub-cultured from -80˚C on to CARBA-SMART agar, incubated aerobically for 24hrs and transported using charcoal swabs to the University of Oxford for DNA extraction prior to long read SMRT sequencing at Icahn Institute for Genomics and Multiscale Biology (NY, USA). At Oxford, the isolates were re-plated on to Columbia blood agar (CBA) (Oxoid, UK) impregnated on one side with three ERT (Fisher Scientific, USA) 10 µg discs and incubated for 18 hours at 37˚C. A 10µl loop of pure culture was used to transfer each isolate to a lysis buffer solution containing 3.5ml buffer B1 (Qiagen, Germany), 80µl lysozyme (100 mg/ml) (Sigma-Aldrich, UK), 7µl RNAse (100 mg/ml) (Sigma-Aldrich, UK)) and 80µl proteinase K (Qiagen, Germany). The sample was vortexed until homogeneous and incubated at 37˚C for 30 minutes. To each sample, 1.2 ml of buffer B2 (Qiagen, Germany) was added and incubated at 50˚C for 30 minutes followed by centrifuging at 4000 rpm for 10 minutes at 4˚C (5810 R, Eppendorf, UK). A genomic tip 100/G (Qiagen, Germany) was equilibrated with 4 ml buffer QBT (Qiagen, Germany), followed by the addition of the sample supernatant and left to pass through the column by gravity flow. DNA was eluted from the column with 5ml buffer QF (Qiagen, Germany) by gravity flow, after two washing steps with 7.5ml buffer QC (Qiagen, Germany). DNA was precipitated with 3.5 ml isopropanol (Fisher Scientific, USA) and centrifuged at 4000 rpm

for 15 minutes at 4˚C (5810 R, Eppendorf, UK). Following supernatant removal, the pellet was washed with 2 ml 70% ethanol (Fisher Scientific, USA) chilled at 4˚C. The sample was centrifuged at 4000 rpm for 10 minutes at 4˚C (5810 R, Eppendorf, UK) and the supernatant removed again. The pellet was left to dry (10 minutes at room temperature), then DNA was eluted in CDT-01 elution buffer (Kurabo, Japan) for 2 hours at 55˚C and stored at 4˚C.

DNA quantity

The DNA concentration was assessed using Qubit® Broad Range kit (Invitrogen, UK). Briefly, a stock of reagent buffer was made using a ratio 1 µl of Qubit® dsDNA BR reagent to 199 µl Qubit® BR buffer (both Invitrogen, UK), 199 µl of the reagent buffer added to 1 µl each DNA sample and incubated at room temperature for 2 minutes. DNA concentration was measured using a Qubit® 2.0 fluorimeter (Invitrogen, UK).

DNA quality

DNA quality and fragment length was assessed using the TapeStation (Aligent, USA). Briefly, 1 µl of sample DNA was added to 10 µl Genomic DNA sample buffer (Aligent, USA), vortexed for 20 seconds and centrifuged for 10 seconds at 1544 rpm (5430 Eppendorf, UK). Each sample was run on a TapeStation 2200 (Aligent, USA) using a Genomic DNA Screentape (Aligent, USA) and the fragment size and DNA integrity number (DIN) was determined (supplementary table 2).

Table S1. Approximate numbers of CPE added to the model

Day	**Dilution**	**Approximate inocula**
15	10^{-7}	1.90 $\log_{10}$ cfu/mL
16	10^{-6}	2.90 $\log_{10}$ cfu/mL
17	10^{-5}	3.90 $\log_{10}$ cfu/mL

18	10^{-4}	4.90 $\log_{10}$ cfu/mL
19	10^{-3}	5.90 $\log_{10}$ cfu/mL
20	10^{-2}	6.90 $\log_{10}$ cfu/mL
21	10^{-1}	7.90 $\log_{10}$ cfu/mL
22	neat	8.90 $\log_{10}$ cfu/mL

Sequencing

Genomic DNA library preparation and sequencing was performed primarily using the manufacturer's instructions and reflects the P6-C4 sequencing enzyme and chemistries. In short, ~5 µg of extracted, high-quality, gDNA was quantified and diluted to 150 µL using Qiagen elution buffer at 33 µg / µL and then sheared to ~20 kbp using a Covaris G-tube spin column and sheared gently for 60 seconds at 4500 rpm using an Eppendorf 5424 bench centrifuge. The sheared DNA was then re-purified using a 0.45X AMPure XP purification step. After purification and shearing, purified and sheared sample was taken into DNA damage and end-repair from each batch preparation. Briefly, the DNA fragments were repaired using DNA damage repair solution (1X DNA damage repair buffer, 1X NAD+, 1 mM ATP high, 0.1 mM dNTP, and 1X DNA damage repair mix) with a volume of 21.1 µL and incubated at 37ºC for 20 minutes. DNA ends were repaired next by adding 1X end repair mix to the solution, which was incubated at 25ºC for 5 minutes, followed by the second 0.45X Ampure XP purification step. Next, 0.75 µM of blunt adapter was added to the DNA, followed by 1X template preparation buffer, 0.05 mM ATP low and 0.75 U/µL T4 ligase to ligate (final volume of 47.5 µL) the SMRTbell adapters to the DNA fragments. This solution was incubated at 25ºC overnight, followed by a 65ºC 10-minute ligase denaturation step. After ligation, the library was treated with an exonuclease cocktail to remove un-ligated DNA fragments using a solution of 1.81 U/µL Exo III 18 and 0.18 U/µL Exo VII, then incubated at 37ºC for 1 hour. Two additional 0.45X Ampure XP purifications steps were performed to remove < 2000 bp molecular weight DNA and organic

contaminant.

Upon completion of library construction, samples were validated as ~20 kbp using an Agilent DNA 12000 gel chip. Once the P6-C4 sequencing was conducted, Blue Pippin size selection was deployed and the library was sufficient for additional size selection to reduce the portion of library molecules < 7,000 bp. This step was conducted using Sage Science Blue Pippin 0.75% agarose cassettes to select library in the range of 7,000 bp – 50,000 bp. This selection is necessary to narrow the library distribution and maximize the SMRTbell sub-read length for the best *de novo* assembly possible. The resulting library was taken into primer annealing and DNA sequencing on the PacBio RSII machine. Then, primer was annealed to the size-selected SMRTbell at a ratio of 20X with the full-length libraries (80ºC for 2 minute followed by decreasing the temperature by 0.1º/s to 25Cº). The polymerase-template complex was then bound to the P6 enzyme using a ratio of 10:1 polymerase to SMRTbell at 0.5 nM for 4 hours at 30ºC and then held at 4ºC until ready for magbead loading, prior to sequencing. The magbead-loaded, polymerase-bound, SMRTbell libraries were placed onto the RSII machine at a sequencing concentration of 50 pM and configured for sequencing. Data was then generated and assembled using the HGAP3 v2.3.0 SMRTportal assembly pipeline.