

Supplementary Materials

Intensive infection control responses and whole genome sequencing to interrupt and resolve widespread transmission of OXA-181 *Escherichia coli* in a hospital setting

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Supplementary Methods:

Broth microdilutions:

Isolates were subbed from beads stored at -80°C on to Horse Blood Agar (HBA, BioMerieux). Following incubation at 35°C O₂ for 24 hours, single colonies were then inoculated into sterile saline and adjusted to a Macfarland concentration of 0.5. A final concentration of 1 x10⁵ colony forming units/ml was produced by adding the solution to sterile Mueller Hinton Broth (MHB, Thermo Fisher). 50 µl was added to each well in the Sensititre plate. The plates were read using the Sensititre manual viewer, with growth recorded as turbidity or as a deposit of cells at the bottom of the well following incubation at 35°C O₂ for 24 hours. The MIC was interpreted as the lowest concentration of an antimicrobial that inhibits visual growth. All Sensititre plates were assessed for growth control and the inoculum was assessed for purity and colony counts.

Genomic DNA extraction:

The initial six suspected carbapenemase-producing *E. coli* isolates were grown on horse blood agar at 37°C overnight. 2 ml of Luria Bertani (LB) media was added to the plate to resuspend the bacterial growth. 300 µl of resuspension was pelleted and used for DNA extraction using the MoBio UltraClean Microbial DNA extraction kit, as per manufacturer's instructions. All other isolates were grown on horse blood agar at 37°C overnight, and DNA was extracted using the DSP DNA Mini Kit on the QIASymphony SP (Qiagen).

Illumina sequencing:

The initial six isolates were sequenced at the Australian Centre for Ecogenomics (ACE). All subsequent isolates were sequenced at Queensland Forensic and Scientific Services

(QFSS). All libraries were prepared using the Nextera XT DNA preparation kit (Illumina) and sequencing was performed on a NextSeq 500 (Illumina) with 2x150bp chemistry, NextSeq Midoutput kit v2.5.

Nanopore MinION sequencing:

1.5 µg of DNA for MS14441, MS14442 and MS14443 (extracted using the MoBio UltraClean Microbial DNA Kit) was used as input for sequencing on an Oxford Nanopore MinION. Libraries were prepared using the 1D sequencing by ligation (SQK-LSK108) kit (without multiplexing) and loaded onto FLOW-MIN106 R9.4 flow cells. MS14441 and MS14443 were run for 21 and 23 hours, respectively. MS14442 was only run for 14 hours due to flow cell failure.

Supplementary Results:

Nanopore sequencing to fully characterize three representative ST38 isolates

In addition to the IncX3 plasmid, all isolates also contained a ~106.8 kb IncI1 plasmid (Supplementary Figure 3). The closest publicly available plasmids were from *Salmonella enterica* subsp. *enterica* serovar Typhimurium (isolated in the USA in 2007 [USDA-ARS-USMARC-1898, GenBank: CP014972.2] and 2010 [USDA-ARS-USMARC-17823, GenBank: CP014662.1]) and from *E. coli* (isolated in China in 2015 [WCHE050613, GenBank: CP019215.2]). No resistance or virulence genes were identified on the plasmid, nor were there any genes associated with metabolic activity based on *in silico* analysis. Further work is required to determine the benefit or function, if any, of this plasmid.

All three isolates sequenced with Nanopore MinION (MS14441, MS14442 and MS14443) had operons relating to adhesion and biofilm formation (*csg*, *ecp*, and *fim*), iron uptake (*chu*, *ent*, *fep*, *sil*) and secretion (including type II [*gsp*], and a putative type VI [*aec*]). Presence of the *E. coli* type III secretion system 2 (ETT2) was confirmed in all three, however the locus of enterocyte effacement (LEE) was absent. All isolates carried the same prophage regions throughout their chromosome except that MS14441 had a single additional ~32 kb phage absent from MS14442 and MS14443. No other genomic rearrangements (large inversions, deletions or insertions) in the chromosome or plasmids were detected.

SNPs and structural variants in SS17M6399 and SS17M6415

Isolate SS17M6399 was found to have 264 SNPs within a ~9.6 kb region corresponding to a phage tail protein. On closer inspection, this appeared to be caused by mis-mapping of reads from a similar prophage in SS17M6399. PHASTER analysis identified additional

prophage sequences in this genome compared to the reference strain MS14441 (Supplementary Table 2 and Supplementary Figure 4). Isolate SS17M6415 appeared to have a different IncI1 plasmid (albeit with a similar IncI1 plasmid backbone) compared to the majority of outbreak isolates (Supplementary Figure 6).

Acquisition of different plasmids in three isolates

Three isolates, SS17M5159, SS17M5157 and SSM6408, all had additional IncQ, IncL/M and IncI2 plasmid types, respectively. Acquisition of the IncQ plasmid in SS17M5159 corresponded to additional resistance genes, including *tetA*, *tetR*, *bla*_{TEM-1b}, *dfrA5* and *sul2*. These additions are predicted to confer resistance to streptomycin, sulphonamides and tetracycline, consistent with the antibiogram for this isolate. Comparison of this IncQ plasmid to publicly available genomes identified a similar ~115 kb plasmid (pO111-CRL-115, GenBank KC340959.1) isolated in Australia between 1999-2002 (Supplementary Figure 8 and 9).

Supplementary Tables:

Supplementary Table 1: Predicted consequences of SNPs in Supplementary Figure 1

Isolate	SNP location (bp) relative to MS14445^a reference	Description
MS14442	615162	Results in stop codon (TAA) in gene for OmpF outer membrane porin (linked with carbapenem resistance)
	987790	D (GAC) -> Y (TAC) in gene for OmpC
	989792	P (CCT) -> S (TCT) in gene for Phosphotransferase RcsD
	2716928 (plasmid)	Single SNP upstream of bla _{OXA-181} (GGGGACGTTATG -> GGGGG <u>G</u> CTTATG)
MS14445	2716928	G (GGC) -> G (GGT) in gene for putative FAD-linked oxidoreductase

^a Original concatenated *de novo* illumina assembly of *E. coli* ST38 strain MS14445 (see additional file for unconcatenated version)

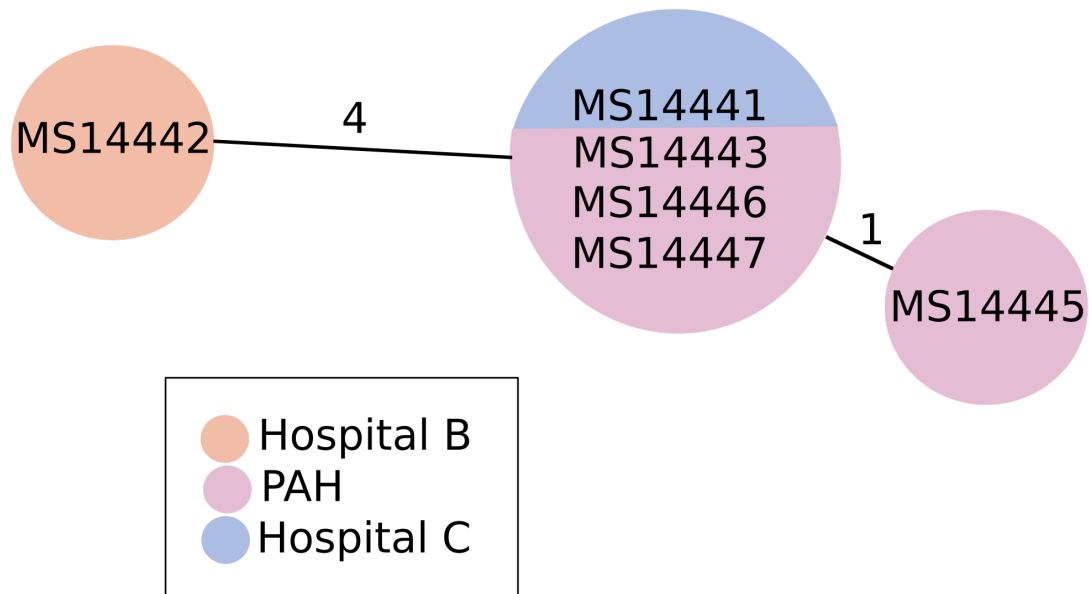
Supplementary Table 2: PHASTER comparison of phage sequences in SS17M6399 compared with draft Nanopore reference strain MS14441

Phage	SS17M6399	MS14441	pMS14441A	pMS14441B
EnteromEp460_NC_019716(10)	49.16%/ 43.94%	47.35%		
Vibro_12B12_NC_021070(27)		52.42%		
Escher_pro483_NC_028943(35)	45.69%	51.02%		
Enter_933W_NC_000924(2)	47.48%	47.05%		
Shigel_Stx_NC_029120(5)	47.33%			
Enterolambda_NC_001416(20)	50.83%			
Enter_N15_NC_001901(2)	51.68%		56.56%	
Stx2_c_1717_NC_001357(2)	43.33%			
Cronob_vB_CsaM_CAP32_NC_019401(1)	48.50%			
Klebsi_phiKO2_NC_005857(2)			54.03%	
Staphy_SPbeta_like_NC_029119(2)				49.74%/ 50.11%

Supplementary Table 3: SNP consequences in blood isolate M94949

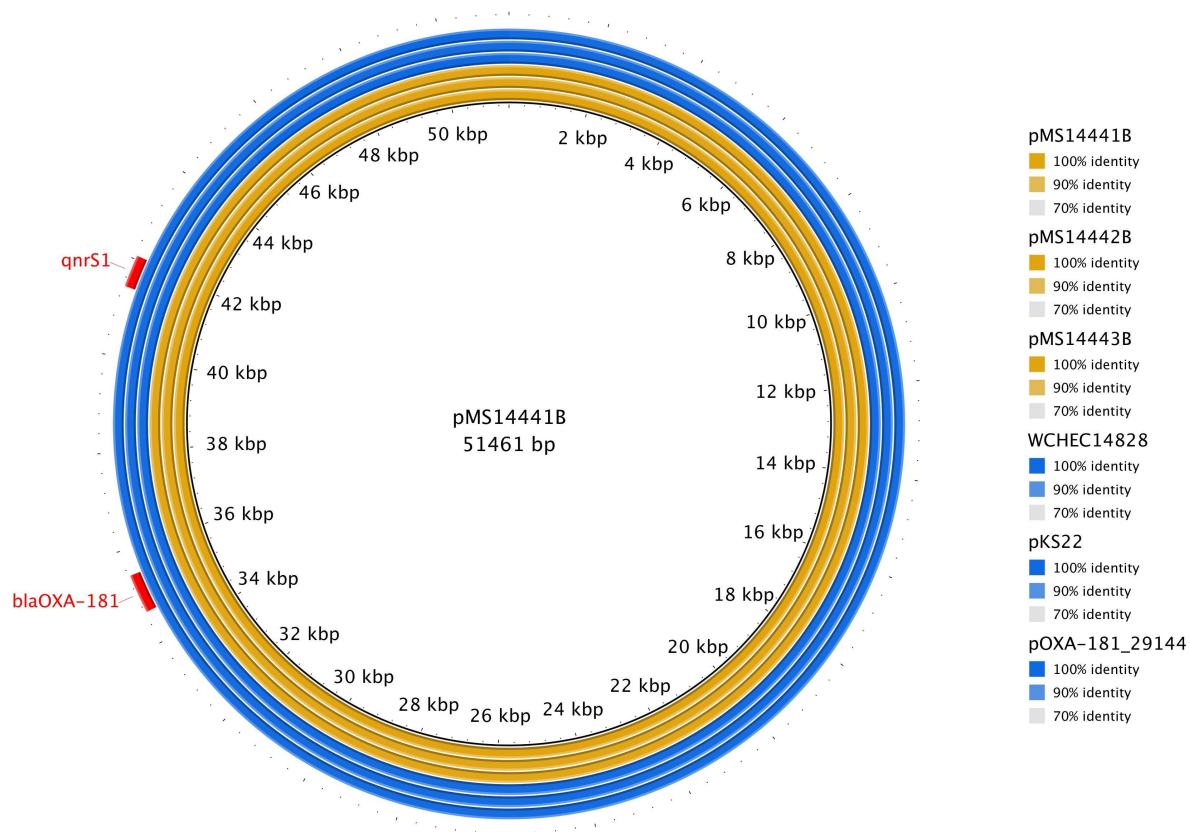
SNP location (in MS14445 reference)	Type	Description
78591	Non-synonymous	Non-synonymous SNP in <i>prc</i> gene (involved in cleavage of C-terminus of penicillin-binding protein 3 (PBP3))
295377	Synonymous	Within <i>uidABC</i> system, involved in transport of glucuronides
1360125	Non-synonymous	In <i>folA</i> gene, involved in folate metabolism
1641057	Non-synonymous	In <i>puuA</i> gene, involved in utilization of putrescine as carbon and nitrogen source
2134299	Non-synonymous	Uncharacterised protein YqeB
2192206	Non-synonymous	In <i>epd</i> gene, catalyses NAD-dependent conversion of D-erythrose 4-phosphate to 4-phosphoerythronate
3372972	Synonymous	In DEAD-box RNA helicase gene involved in RNA degradation
3658694	Non-synonymous	Required for induction of expression of the formate dehydrogenase H and hydrogenase-3 structural genes
4546526	Intergenic	Does not appear to be within promoter region of surrounding genes
4678640	Synonymous	In <i>fdnG</i> gene, enables <i>E. coli</i> to use formate as major electron donor during anaerobic respiration
4679448	Non-synonymous	

Supplementary Figures:



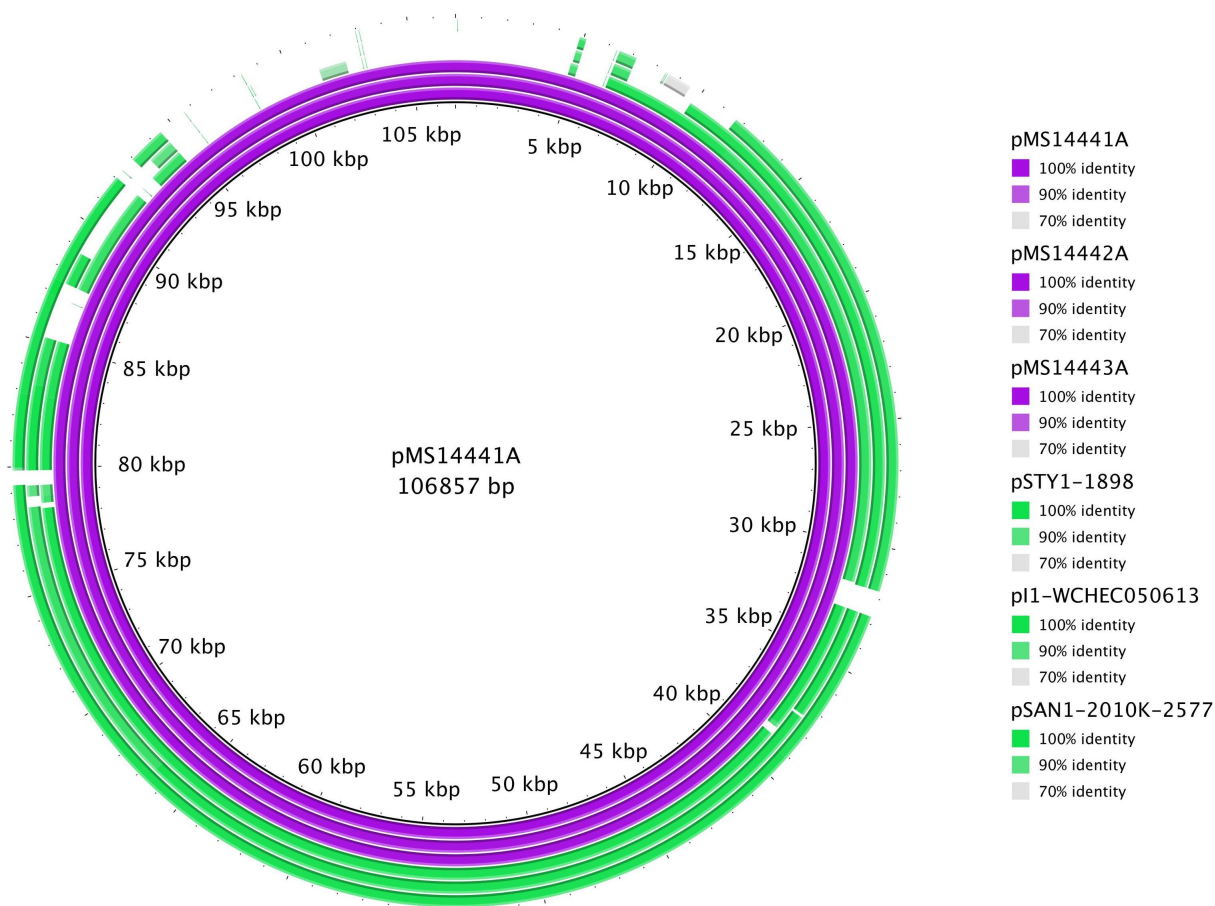
Supplementary Figure 1: Relationship matrix of initial 6 *E. coli* isolates

Numbers indicate core SNP distances between isolate genomes. Isolate genomes sharing the same circle are identical at the core SNP level.



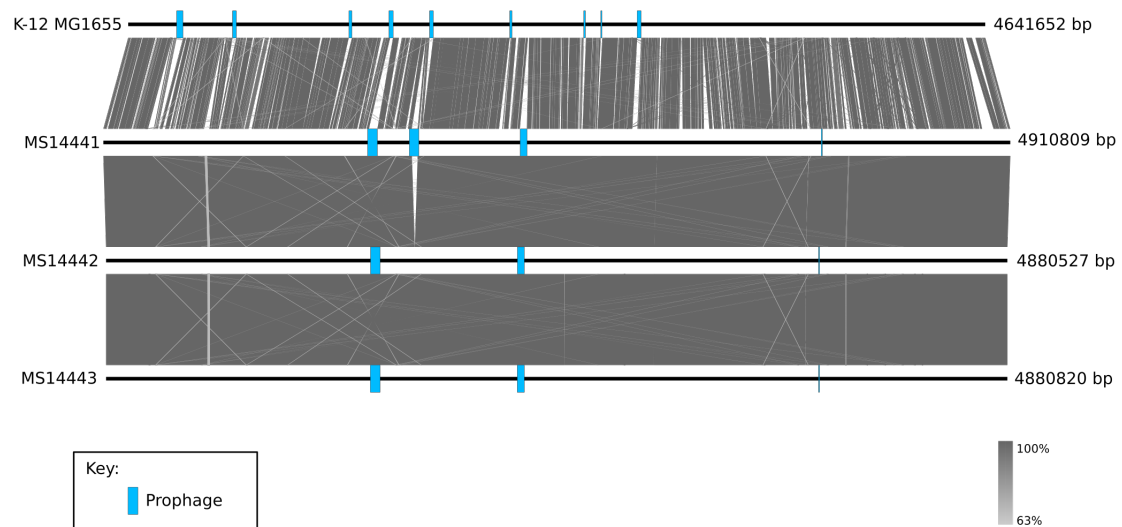
Supplementary Figure 2: BRIG comparison of Nanopore IncX3 plasmids and publicly available plasmids

BLAST Ring Image Generator (BRIG) implementing unfiltered BLASTn with individual query sequences listed in legend on right. Antibiotic resistance genes are highlighted in red.



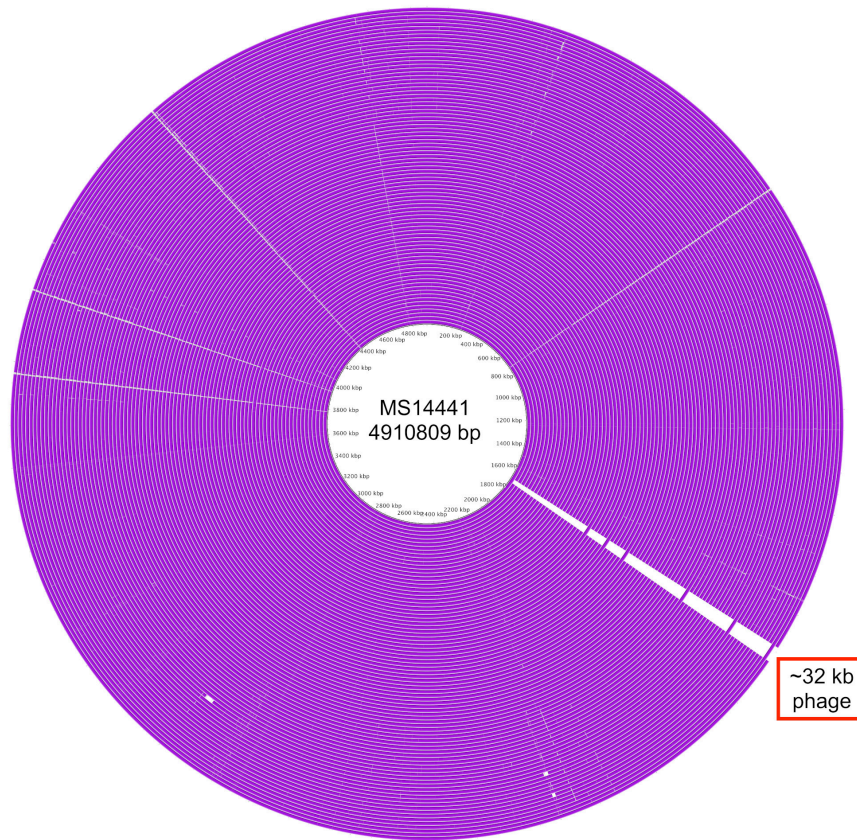
Supplementary Figure 3: BRIG comparison of Nanopore Inel1 plasmids and publicly available plasmids

BLAST Ring Image Generator (BRIG) implementing unfiltered BLASTn with pMS14441A as reference subject sequence and individual query sequences listed in legend on right (innermost ring listed at top of legend).



Supplementary Figure 4: Comparison of entire chromosomes of K-12 MG1655, MS14441, MS14442 and MS14443

Comparison was carried out with BLASTn as implemented in Easyfig. Prophage regions are annotated in blue.



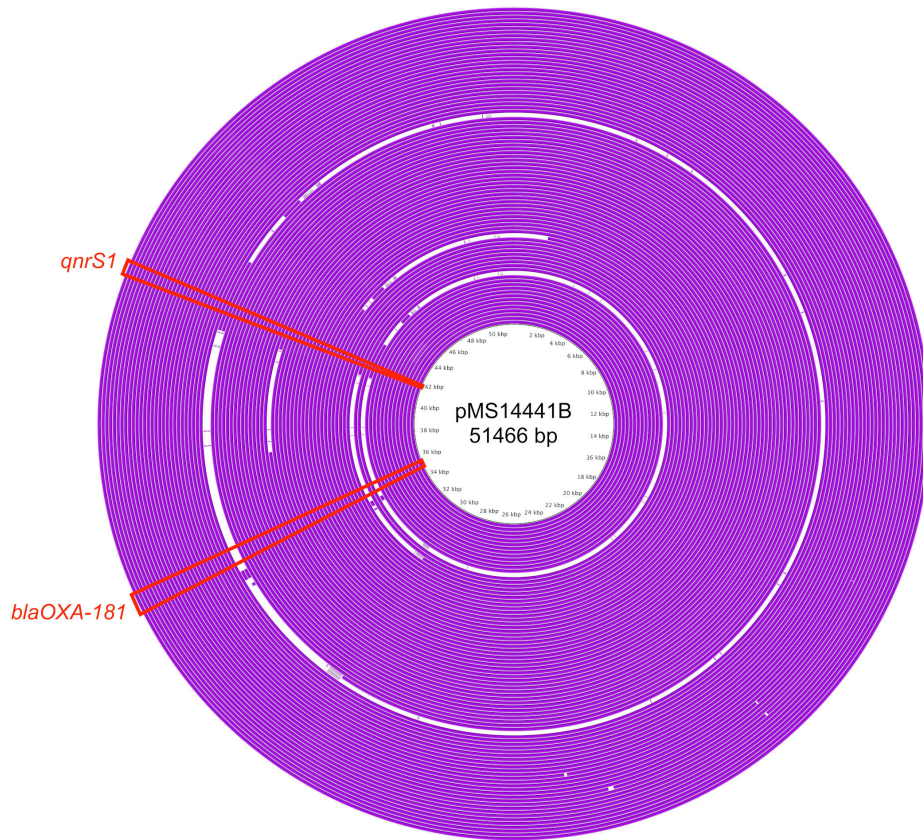
Supplementary Figure 5: BRIG comparison of all ST38 outbreak isolates to MS14441 chromosome

For the list of strains for each ring, see Supplementary Dataset S1 (tab 3).



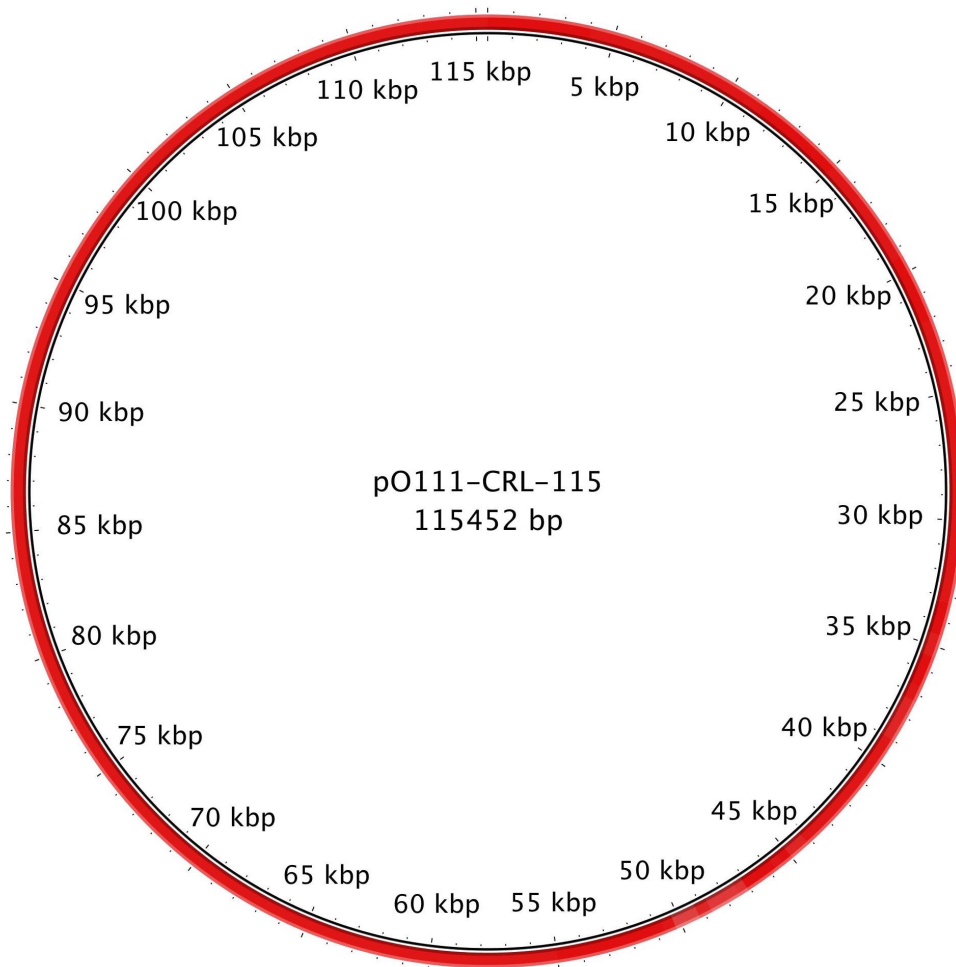
Supplementary Figure 6: BRIG comparison of all ST38 outbreak isolates to pMS14441A (Incl1 plasmid)

All isolates appeared to have a very similar/identical Incl1 plasmid, with the exception of SS17M6415 (second outermost ring), which appears to have a different Incl plasmid, albeit with a similar Incl backbone. For the list of strains for each ring, see Supplementary Dataset S1 (tab 3).

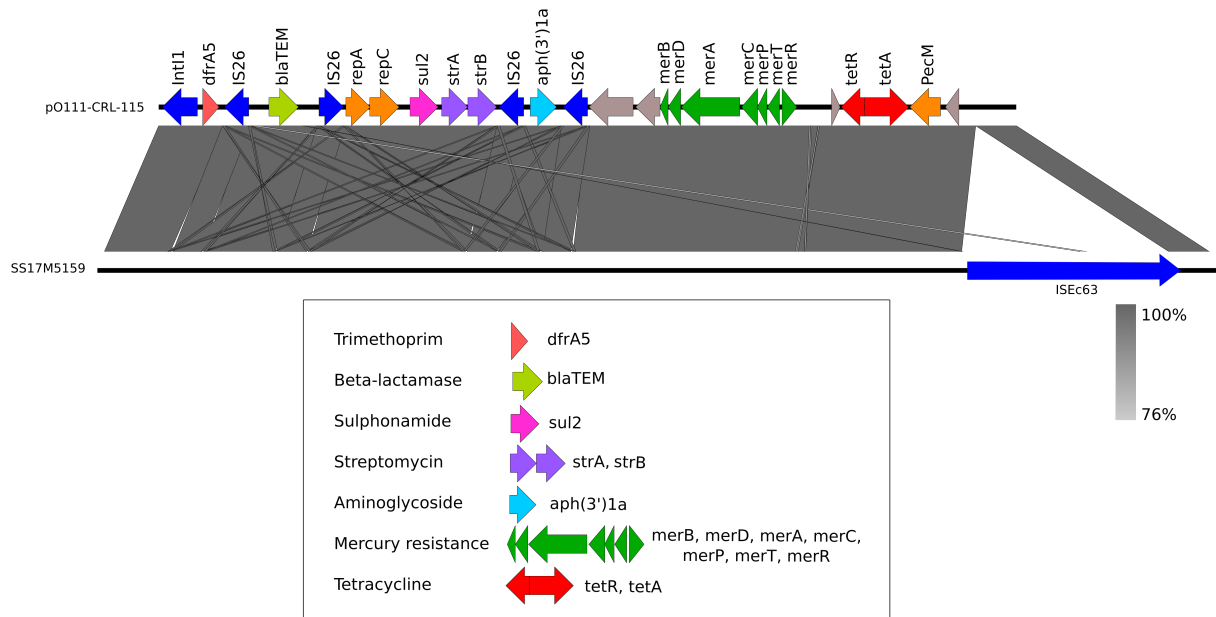


Supplementary Figure 7: BRIG comparison of all ST38 outbreak isolates to pMS14441B (IncX3 plasmid)

For the list of strains for each ring, see Supplementary Dataset S1 (tab 3).

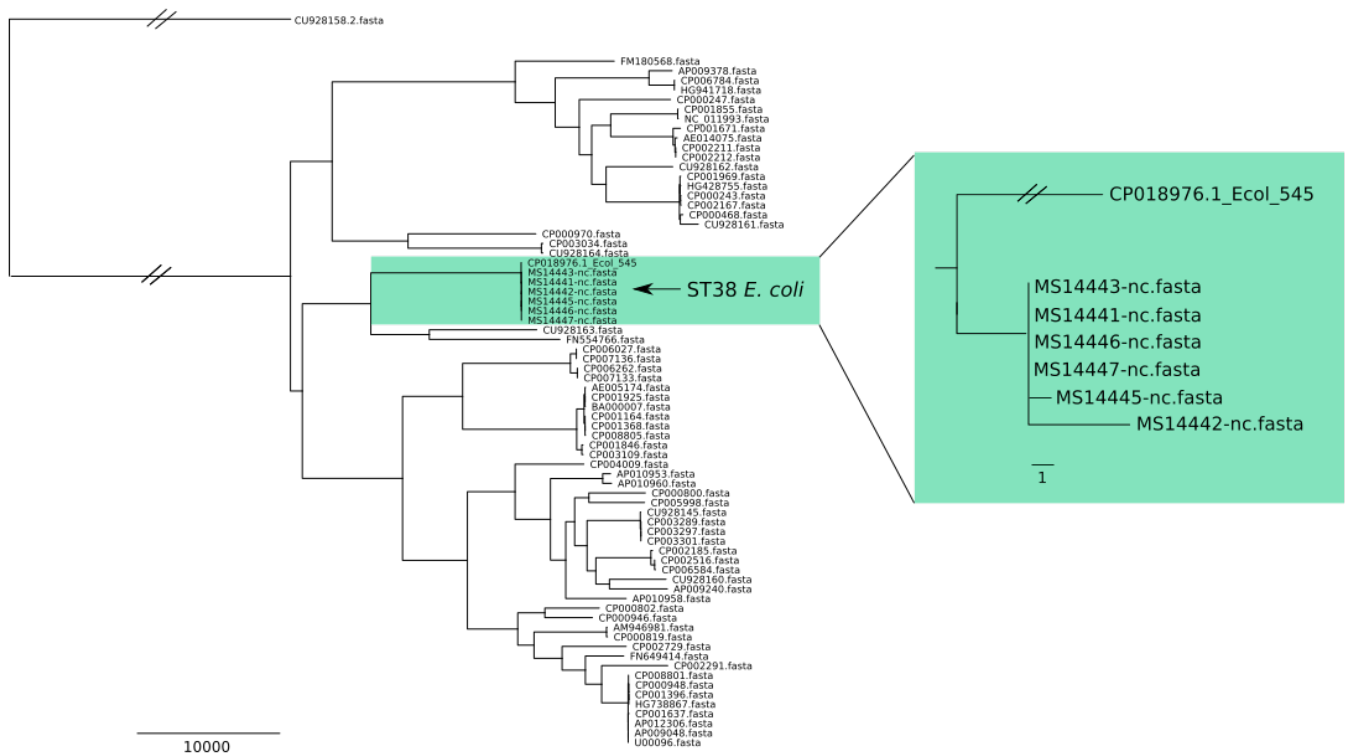


Supplementary Figure 8: BRIG comparison of SS17M5159 draft assembly to pO111-CRL-115



Supplementary Figure 9: Context of antibiotic resistance genes in SS17M5159 based on reference plasmid pO111-CRL-115

BLASTn comparison of pO111-CRL-115 resistance region (top) in comparison with matching plasmid contigs from draft SS17M5159 genome (bottom) prepared using Easyfig.



Supplementary Figure 10: Contextualizing ST38 outbreak isolates against publicly available *E. coli* complete genomes

The MS14441 reference chromosome (generated using Nanopore MinION) was used to search the non-redundant nucleotide (nr/nt) database available on NCBI's using BLAST [<https://blast.ncbi.nlm.nih.gov/Blast.cgi>] (accessed 28th May 2017). *E. coli* 545 (CP018976.1) appeared as the closest match. A selection of available complete *E. coli* genomes were used to contextualize the outbreak isolates.