New kids on the block: Intercontinental dissemination and transmission of newly emerging lineages of multi-drug resistant *Escherichia coli* with highly dynamic resistance gene acquisition.

Zhiyong Zong[1], Feng Yu[1], Alan McNally[2]

[1]Centre for Infectious Diseases, West China Hospital of Sichuan University, Chengdu, China

[2]Institute of Microbiology and Infection, College of Medical and Dental Science, University of Birmingham, Birmingham B15 2TT

Abstract

Background: The increase in infections as a result of multi-drug resistant strains of *Escherichia coli* is a global health crisis. The emergence of globally disseminated lineages of *E. coli* carrying ESBL genes has been well characterised. An increase in strains producing carbapenemase enzymes and mobile colistin resistance is now being reported, but there is little data on emerging global lineages of such strains.

Methods: Routine screening of patients within an ICU of West China Hospital identified a potential outbreak of *E. coli* carrying the $bla_{\text{NDM-5}}$ carbapenemase gene. Genome sequencing was performed on the strains isolated. The MLST lineage of the strains was determined and a global collection of genomes of relevant lineages utilised to characterise the emergence of two globally disseminated carbapenemase-producing Enterobacteriaceae (CPE) lineages of *E. coli*.

Findings: Our data describes the presence of *E. coli* ST167 and ST617 in a potential West China Hospital outbreak, which are globally disseminated CPE lineages of *E. coli*. Both lineages show extreme levels of MDR gene acquisition ranging across ESBL, CPE, and mobile colistin resistance genes. Our analysis also shows real-time switching of CPE genes in a plasmid and real-time transfer of the plasmid across lineages, as well as recent inter-continental transmission of the ST167 lineage.

Interpretation: Our data suggests that dominant lineages of *E. coli* are emerging as CPE clinical threats, in much the same way as occurred for ESBL lineages of *E. coli*.

Funding: This work was funded by a Royal Society Newton Advanced Fellowship project (NA150363) awarded to Zhiyong Zong and Alan McNally and a grant from the National Natural Science Foundation of China (project no. 8151101182) to Zhiyong Zong.

Introduction

Infections from multi-drug resistant (MDR) *Escherichia coli* are a significant global health care threat [1]. Despite being an extremely diverse species, MDR in *E. coli* is largely confined to strains capable of causing extra-intestinal infections (ExPEC) such as urinary tract infections (UTI) and bacteraemia [1–4]. Over 50% of *E. coli* strains isolated globally from UTI and bacteraemia cases exhibit resistance to three or more classes of antibiotic, termed MDR. This resistance phenotype is primarily driven by the acquisition and stable maintenance of large MDR-plasmids containing multiple resistance genes [2]. The rapid global dissemination of MDR *E. coli* is particularly associated with carriage of plasmids containing genes encoding extended-spectrum β-lactamases (ESBL) which confer resistance to third-generation cephalosporins [5]. The carriage of MDR plasmids containing ESBL genes renders *E. coli* susceptible only to the carbapenem class of antibiotics and the last-resort antimicrobial compound colistin [5]. However strains of *E. coli* are now being reported which contain plasmids containing β-lactamases conferring resistance to carbapenems (carbapenemases) and the *mcr-1* gene which confers resistance to colistin [6–9].

The global dissemination of ESBL *E. coli* is attributable to the rapid global dispersal of a small number of *E. coli* lineages. The most dominant of these is the ST131 lineage which is predominantly associated with carriage of the CTX-M-15 type of ESBL gene [2]. ST131 is an ExPEC lineage which is the most common cause of UTI and bacteraemia in the developed world [2]. Other dominant lineages of ESBL *E. coli* are ST73, ST95, and ST648 which are also ExPEC lineages [3,4]. ESBL carriage can also be found transiently in strains belonging the ST10 clonal complex of *E. coli* [3]. ST10 complex strains are host generalist *E. coli* which are frequently found as intestinal commensal inhabitants of mammals and avian species [10], and are devoid

of the virulence-associated genes known to be required for intestinal and extra-intestinal pathogenesis [11]. The epidemiology of ESBL dissemination in *E. coli* is extremely well characterised, however the relatively recent emergence of carbapenemase-producing *E. coli* (CPE) and the *mcr-1* colisitin resistance gene, means that very little population genetic data exists on the dissemination of these resistances in *E. coli*.

Here we report the presence of a local outbreak of *E. coli* containing the carbapenem-resistance gene new-Delhi metallo-β-lactamase (NDM-5) in an ICU ward in West China Hospital, Chengdu. Surprisingly the causative isolates do not belong to one of the dominant MDR lineages of ExPEC, but to ST167 and ST617, both members of the ST10 clonal complex. Genomic epidemiology data supports the long-term presence of these bacteria in the ICU with repeated dissemination from a central reservoir. Contextualisation of the Chinese outbreak strains with a global collection of genomes shows the global dissemination of MDR ST167 and ST617 strains, and in the case of ST167 highly dynamic acquisition of ESBL and carbapenemase genes. And in the case of ST167 we provide evidence for switching of the carbapenemase gene type in the MDR plasmid during the outbreak, and then intercontinental transmission of the switched variant from China to the USA. Our data identify two emerging lineages of MDR *E. coli* which present a significant global health care threat.

Methods

Bacterial isolation and characterisation

Strain 0215 was recovered from a rectal swab of a 75-year-old male patient on September 2013 in a 50-bed medical ICU at West China Hospital, Chengdu, China, during a preliminary screening project. Following the identification of $bla_{\text{NDM-5}}$, we

performed an active screening project on adult patients (age ≥16) at the medical ICU ward during a 7-month period from May to November 2014. This study was conducted in accordance with the amended Declaration of Helsinki and was approved, under a waiver of consent, by the Ethics Committee of West China Hospital. Rectal swabs were collected from patients within 2 days of admission to the ICU and within the 3 days prior to ICU discharge for those patients with a length of stay of 3 days or more. Swabs were transferred to the laboratory in transport media and were screened for carbapenem-resistant Enterobacteriaceae using the CHROMAgar Orientation agar plates (CHROMAgar, Paris, France) containing 2 μg/ml meropenem. Carbapenem-resistant *E. coli* were recovered from the rectal swabs of 8 different patients (Table 1). Furthermore, one of the 8 patients developed bacteraemia during his ICU stay and an *E. coli* was recovered from his blood and included in the study. During the study period, two additional *E. coli* clinical isolates carrying $bla_{\text{NDM-5}}$ were recovered in the hospital. Both isolates were recovered on admission to the hospital from two different patients.

Genome sequencing

The ST167 and ST617 strains isolated in Chengdu were cultured in LB broth at 37 degree Celsius overnight. DNA was extracted using QIAamp® DNA Mini Kit (QIAGEN) and 150 bp paired-end library of each strain prepared and sequenced using the Illumina HiSeq X Ten platform (raw data accession numbers Table S1 and S2). Genomes were assembled using SPAdes [12] with the --careful flag and annotated using Prokka [13]. The MLST sequence type of the strains was determined using the in silico MLST prediction tool MLSTFinder [14]. The *E. coli* genome database Enterobase (www.enterobase.warwick.ac.uk) was interrogated on 1st December 2016 and all available ST167 and ST617 genomes were downloaded (Table S1 and

S2) and annotated using Prokka. The antibiotic resistance gene profile of all isolates was determined using Abricate (https://github.com/tseemann/abricate), and the plasmid replicon profile and virulence-associated gene profile determined using the PlasmidFinder and VirulenceFinder databases [15].

Phylogenetic analysis

Separate pan-genomes were constructed for the ST167 and ST617 datasets using Roary [16] with the --e --mafft setting to create a concatenated alignment of core CDS. The alignments were used to infer ST167 and ST617 phylogenies using RaxML [17] with the GTR-Gamma model of site heterogeneity and with 100 bootstrap iterations. Carriage of MDR beta-lactamase genes was annotated on the trees using Phandango (https://jameshadfield.github.io/phandango/), and geographical source was annotated onto the trees using iTOL [18].

High-resolution SNP outbreak analysis

The ST167 genomes associated with the Chengdu outbreak were analysed by mapping raw reads against the *E. coli* MG1655 (ST10) reference genome (Accession number: NC_000913.3). Maping was performed using bowtie2 [19] and the resulting alignment processed with samtools and vcffilter to keep only SNPs with a minimum quality of 30, a minimum read depth of 10, and a minimum allele frequency of 0.9 [20]. Indels were also removed, and SNPs located in mobile elements such as IS elements, transposons, and phages were also removed. This final SNP profile was used to create a consensus sequence for each genome which was aligned using the parsnp alignment tool in Harvest [21]. Analysis of the resulting alignments identified multiple large scale recombination events in the ST167 genomes relative to MG1655, and so these were also removed using Gubbins [22] to provide a final high-resolution SNP profile of the outbreak.

Plasmid sequence analysis

The *bla*$_{NDM-5}$ containing plasmid from ST167 strain 0215, designated pNDM5 was recovered by conjugational transfer, using *E. coli* strain J53 as the recipient and 4 μg/ml meropenem plus 150 μg/ml sodium azide as the selective antibiotics. A complete plasmid sequence was obtained by circularizing plasmid fragments assembled from Illumina reads using conventional PCR and Sanger sequencing. Further analysis on the complete sequence of pNDM5_0215 revealed that it was a 47-kb IncX3 plasmid and there were no antibiotic resistant genes other than *bla*$_{NDM-5}$ located on the same plasmid [23]. The resulting plasmid sequence was used as a reference to align contigs from the ST167 outbreak strains and ST617 Chengdu strains by pairwise Blastn using BRIG [24]. Comparison was also made with the *bla*$_{OXA-181}$ containing plasmid previously isolated from Chengdu [25].

Role of the funding source

The authors confirm that the project funders played no role in study design, the collection, analysis, and interpretation of data, the writing of the report, or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Presence of *E. coli* ST167 and ST617 strains containing the NDM-5 carbapenemase resistance gene in an ICU ward in West China Hospital.

A total of ten isolates of *E. coli* containing *bla*$_{NDM-5}$ were obtained during the investigation. Nine of these isolates belonged to sequence types ST167/617, which are members of the ST10 complex of *E. coli* most commonly associated with

mammalian intestinal commensal carriage. Three ST167 isolates (0215, 243 and 25) were obtained from swabs or clinical samples collected on admission to hospital, suggesting that they were introduced from external sources. The three patients were all citizens of Chengdu city but they were admitted to different local hospitals before transferring to West China hospital. The remaining ST167 isolates were recovered from swabs or samples collected at least 3 days after admission to the ICU of West China hospital, suggesting that they were acquired during their ICU stay. ST167 *E. coli* carrying $bla_{\text{NDM-5}}$ caused infections (bacteremia and abdominal infection) in only two patients but colonized the remaining patients. Both ST617 *E. coli* carrying $bla_{\text{NDM-5}}$ caused colonization. All patients colonised or infected with *E. coli* carrying $bla_{\text{NDM-5}}$ of ST167 or ST617 had received carbapenems before the recovery of the isolates.

ST167 and ST617 are both globally disseminated lineages of MDR *E. coli* showing frequent independent acquisition of carbapenem resistance.

We sought to contextualise our Chengdu isolates by obtaining a wider collection of ST167 and ST617 genomes. We searched the Enterobase *E. coli* database and recovered a total of 87 genomes of ST167 (table S1) and 86 genomes of ST617 (table S2), isolated from across the world. A core CDS-based phylogeny of both lineages showed a diverse set of genomes with around 17,000 SNPs in ST167 and around 15,000 SNPs in ST617. Annotation of the ST617 phylogeny with beta-lactamase gene carriage showed a striking pattern of carriage of the $bla_{\text{CTX-M-15}}$ ESBL gene (Fig 1A) suggesting a clonal expansion in the lineage associated with $bla_{\text{CTX-M-15}}$ carriage as in the pandemic *E. coli* ST131 lineage [26,27]. Annotation of the ST167 phylogeny with β-lactamase gene carriage (Fig 1B) shows a very different

pattern of emergence, with multiple independent acquisition of carbapenemase across the phylogeny including bla_{NDM-1}, bla_{NDM-5}, bla_{NDM-7}, $bla_{OXA-181}$, and bla_{KPC-3}. For both phylogenies there is clear evidence of inter-continental movement of bacteria, with geographical source being randomly distributed across both phylogenies (Fig S1, Fig S2). Analysis of the ST167 phylogeny confirmed the presence of the Chengdu isolates as a monophyletic cluster, but nestled within the outbreak is a genome downloaded from Enterobase, genome DHQPCR312 (Fig 2). Recovery of the Biosample data for this strain from the NCBI SRA archive shows that this strain was isolated from a urine sample in New York state, USA and was deposited into NCBI in January 2016. Curiously this strain does not contain NDM-5 but rather contains $bla_{OXA-181}$ and $bla_{CTX-M-11}$.

SNP analysis confirms inter-continental transmission of an MDR *E. coli* ST167 outbreak.

We sought to investigate the unexpected appearance of a US isolate with altered MDR gene carriage in the middle of our Chengdu outbreak phylogeny. We obtained the raw fastQ sequence data for the US strain DHQPCR312 from the NCBI SRA archive (Accession number: SRR3222296) and mapped reads of all the outbreak strains against *E. coli* MG1655, which is an ST10 strain. The resulting alignments were filtered to leave only high-resolution SNPs, with nucleotide distances in general agreement with those obtained from the cruder assembly-derived core CDS alignment (dataset S1). The high-resolution SNP phylogeny of the outbreak shows three distinct branching events, each separated by 50-100 SNPs (Fig 3), despite isolation dates in the three groups overlapping. The data also shows that the US isolate DHQPCR312 is also clearly connected to the Chengdu ICU outbreak despite

having a different MDR gene carriage profile. Such an observation is not consistent with sustained patient-to-patient transmission in the affected ICU [28]. Rather it is consistent with the continued transmission of the strain into patients in the ICU from a central reservoir [28,29].

Plasmid analysis identifies rapid gene switching in MDR plasmids of *E. coli* ST167.

We sought to determine the reason behind the different MDR gene profile in the US strain DHQPCR312 compared to the Chengdu isolates. Crude mapping of the US strain against the Chengdu isolate 0215, and vice versa suggested very little difference in the genomes, even in the plasmid region. We isolated the $bla_{\text{NDM-5}}$ plasmid from strain 0215 by conjugational transfer into an *E. coli* K12 host, and obtained a complete plasmid sequence. We then mapped the contigs of each outbreak isolate to the pNDM5_0215 sequence and visualised using BRIG. Our data shows that the NDM-5 plasmid sequence is present in all the outbreak strains including the DHQPCR312 strain from the US. However in the US strain there is deletion in the region that contains the $bla_{\text{NDM-5}}$ gene (Fig 4A). We noted that a $bla_{\text{OXA-181}}$ plasmid had been isolated from an unrelated strain (*E. coli* of ST410) in West China Hospital at the same time as this outbreak was being investigated, and had been sequenced by our group [25]. We aligned the $bla_{\text{OXA-181}}$ and $bla_{\text{NDM-5}}$ plasmid sequences and mapped the contigs of DHQPCR312 against the alignment (Fig 4B). Our data clearly shows that the $bla_{\text{OXA-181}}$ plasmid is identical to the $bla_{\text{NDM-5}}$ plasmid, except for a region containing the $bla_{\text{NDM-5}}$ gene being swapped for an $bla_{\text{OXA-181}}$ containing locus. Our data also shows that this is the exact same plasmid as is present in US strain DHQPCR312.

Discussion

By investigating the prevalence of carbapenem-resistant *E. coli* in West China Hospital, we identified two lineages of *E. coli* which present a significant global health-care threat as internationally distributed lineages of MDR *E. coli*. The first lineage is ST617, of which two strains were found in West China hospital carrying the NDM-5 metallo-β-lactamase that confers carbapenem resistance. Analysis of a global collection of ST617 strains showed that this lineage has almost certainly arisen as a result of clonal expansion of a $bla_{\text{CTX-M-15}}$ carrying variant, a phenomenon identical to that for the pandemic MDR *E. coli* ST131 lineage [26]. Whilst the global circulation of a $bla_{\text{CTX-M-15}}$ lineage of *E. coli* is significant this lineage merits enhanced surveillance and monitoring because of its ability to also acquire carbapenem resistance genes. Not only was the $bla_{\text{NDM-5}}$ gene acquired in Chengdu, but $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-3}}$ carbapenem resistance genes have also been acquired in independent episodes in this lineage.

Perhaps more significant is our identification of carbapenem resistance in the *E. coli* ST167 lineage. Our data shows that this is a globally disseminated lineage of *E. coli*, and that is has acquired an alarming array of ESBL and carbapenem resistance genes on multiple independent occasions. Identified in the lineage were $bla_{\text{NDM-}1}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-7}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-48}}$ encoding carbapenem resistance, an arsenal of ESBL gene types including $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{SHV-12}}$, and on one occasion the mobile colisitin resistance gene *mcr-1*. To our knowledge no lineage of *E. coli* has been identified with such a vast array of significant MDR genes present across its population. Even in the pandemic ST131 lineage the majority of MDR genes belong to the $bla_{\text{CTX-M}}$ class [27].

Our plasmid analysis data sheds some light on the dynamic nature with which ST167 and ST617 acquire new resistance determinants. The ST167 and ST617 strains isolated in Chengdu all contained the same plasmid carrying the bla_{NDM-5} resistance gene. Given the phylogenies for both lineages the most parsimonious explanation is that this was acquired in Chengdu possibly in the hospital setting. Recent work tracking plasmid dynamics in a single health care facility would support the possibility that this could have occurred in the West China Hospital [30] and highlights the importance of tracking not just strains but also high-fidelity molecular epidemiology of resistance plasmids in health-care settings. Of more significance is the speed with which the resistance genes within the plasmid switched, with the bla_{NDM-5} gene being swapped for an $bla_{OXA-181}$ cassette. Again such plasmid dynamics have recently reported over a longitudinal study of a health-care facility [30], and our dates of isolation, combined with the overlapping dates in which another lineage was found with the new switched plasmid [25] suggest this new plasmid occurred and was disseminated in the wider hospital environment very quickly.

Finally our data show conclusive evidence of inter-continental transmission of a carbapenem resistant *E. coli* ST167. Though it is clear that globally dominant lineages of MDR *E.coli* such as ST131 frequently move between continents [26,27], to our knowledge no direct inter-continental movement has been reported to date, and not for a carbapenem resistant *E. coli*. Attempts to identify the lab which isolated the strain in New York were unsuccessful, however it has to be expected that the New York patient had either visited Chengdu or had close contact with someone returning from Chengdu. Conclusive evidence that carbapenem resistant lineages of *E. coli* can very quickly be globally disseminated highlights the urgent need for a cohesive and concerted global surveillance system for carbapenem resistant *E. coli*. Our data

also shows the need for not just strain level genomic epidemiology, but also that plasmid molecular epidemiology is required to piece together full epidemiological networks of MDR *E. coli*.

Author contributions

Study conceived by ZZ and AM. Data analysed by FY, AM, and ZZ. Manuscript written by AM, ZZ, and FY.

Declaration of interests

All authors confirm there are no conflicts of interest in the research presented

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Table 1. Sources and patients of *E. coli* isolated in West China Hospital carrying bla_{NDM-5}

Isolate	ST	Collection date	Collection, days after admission to ICU	Source	The host patient	
					Age	Sex
0215	167	2013-09	0	Rectal swab	75	Male
243	167	2014-05	0	Rectal swab	84	Female
442[a]	167	2014-07	7	Rectal swab	39	Male
57[a]	167	2014-07	16	Blood	39	Male
936	167	2014-09	12	Rectal swab	63	Female
1222	167	2014-10	7	Rectal swab	17	Male
1237	167	2014-10	3	Rectal swab	44	Female
25	167	2014-10	0	Ascite	45	Female
784	617	2014-08	0	Rectal swab	82	Male
1037	617	2014-09	12	Rectal swab	85	Male

[a]Isolates 442 and 57 were recovered from the same patient.

Figure 1: Maximum likelihood phylogenetic trees of a global collection of (A) ST617 and (B) ST167 strains. The phylogeny is inferred from an alignment of concatenated core CDS sequences as determined by Roary. The annotation denotes the presence of ESBL and CPE associated beta-lactamases as determined by Abricate.

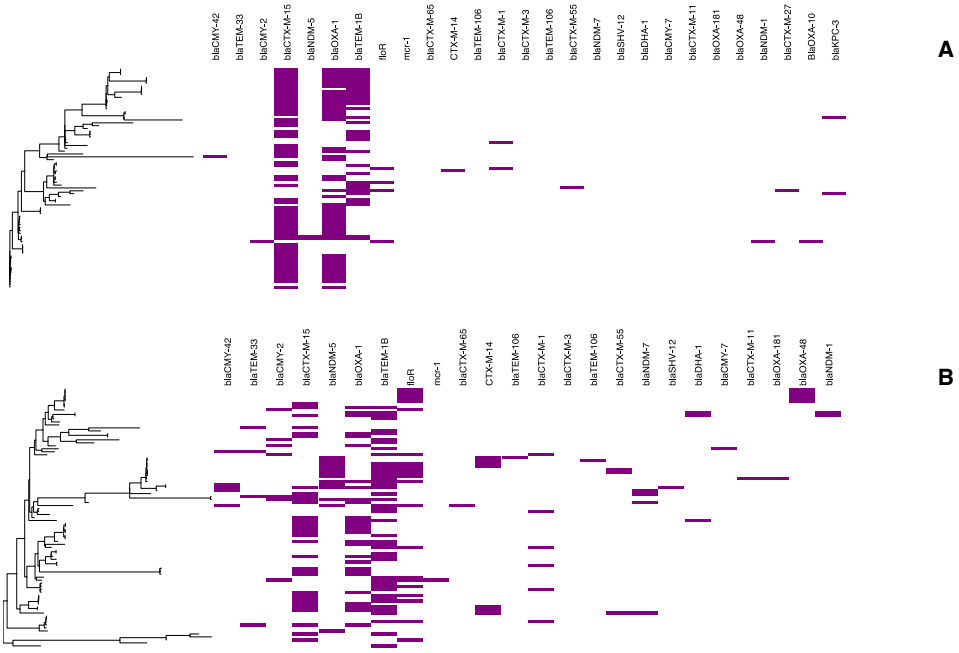

Figure 2: Amplified region of the ST167 maximum likelihood phylogeny containing the West China Hospital outbreak strains. The West China Hospital strains are annotated in red, with the interloping USA strain annotated in blue. The accompanying annotation denotes the presence of ESBL and CPE associated beta-lactamases as determined by Abricate.

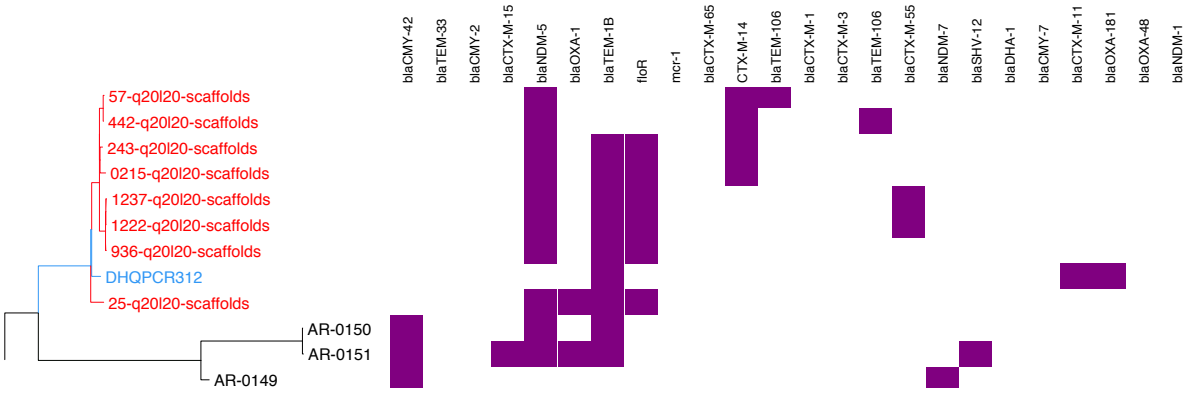

Figure 3: High resolution SNP phylogeny of the ST167 outbreak strains. The phylogeny is inferred from mapping of reads against the MG1655 reference genome, and is unrooted. The annotation denotes the presence of ESBL and CPE associated beta-lactamases as determined by Abricate.

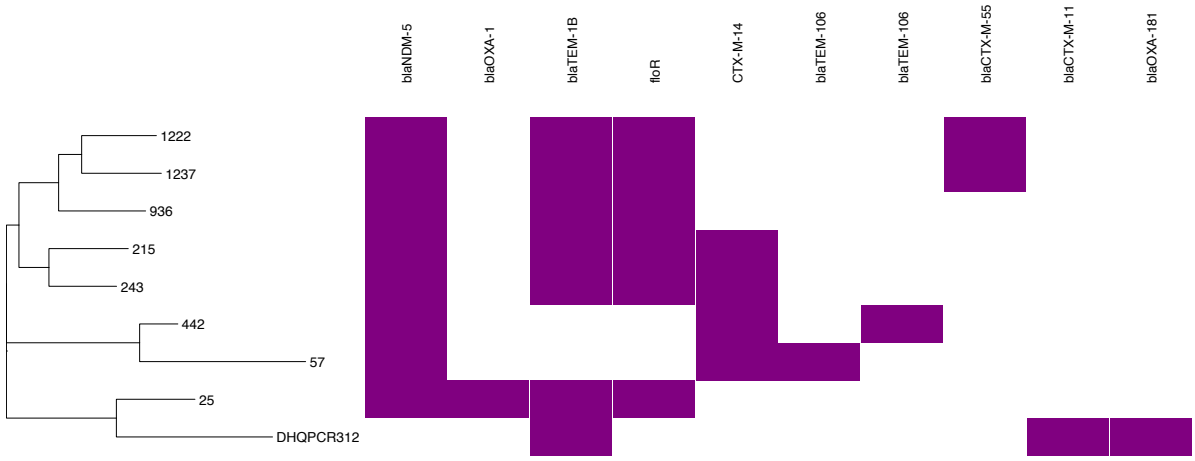

Figure 4: Alignment of contigs from the US ST167 outbreak strain DHQPCR312 using (A) pNDM5 as a reference, and (B) pOXA-181 as a reference. The alignment is a pairwise blastn alignment performed using BRIG.

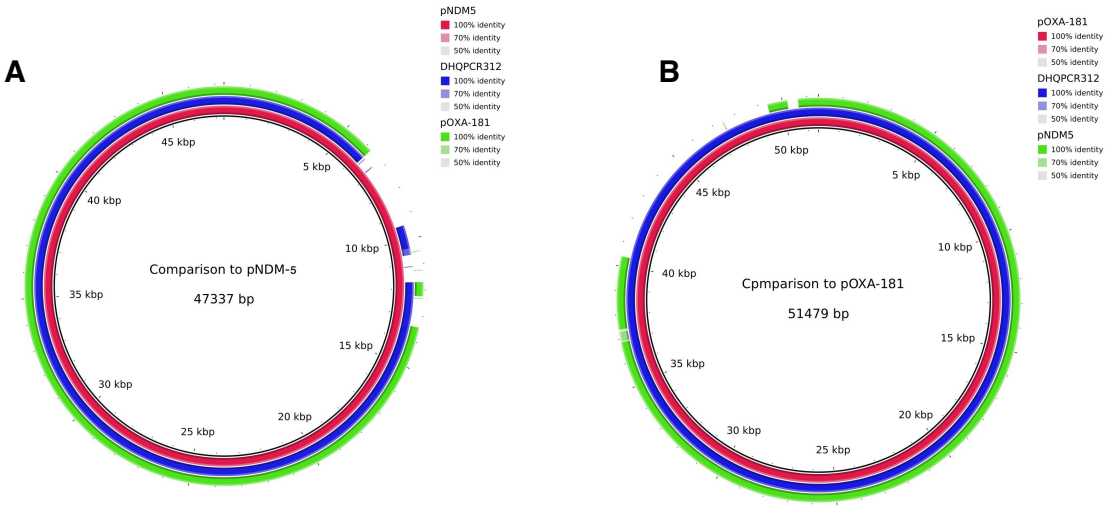

Figure S1: Maximum likelihood phylogeny of a global collection of ST167 strains. The phylogeny is inferred from an alignment of concatenated core CDS sequences as determined by Roary. Annotation denotes the geographical region from which sequenced strains were isolated.

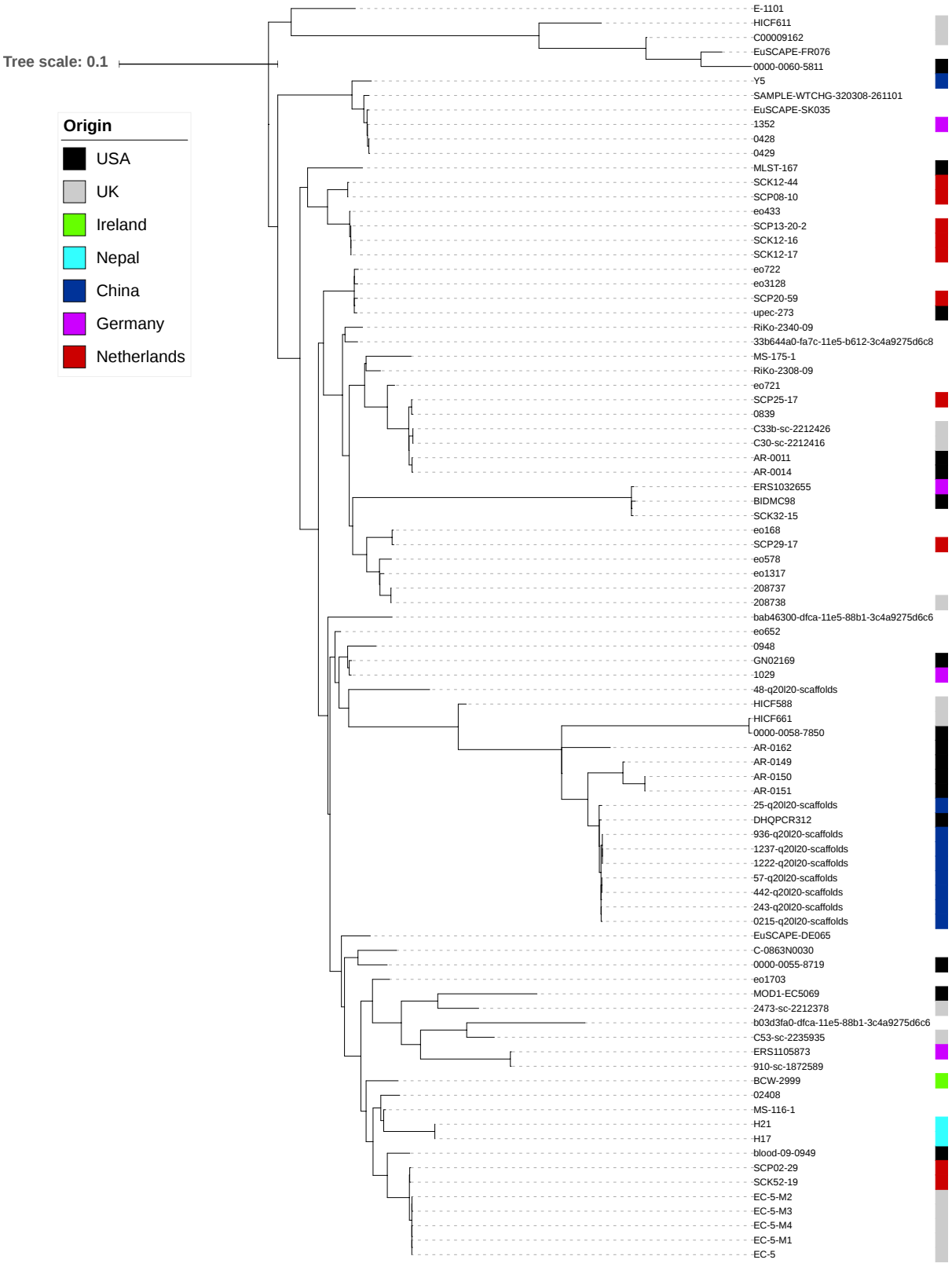

Figure S2: Maximum likelihood phylogeny of a global collection of ST617 strains. The phylogeny is inferred from an alignment of concatenated core CDS sequences as determined by Roary. Annotation denotes the geographical region from which sequenced strains were isolated.