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Genomic comparisons of *Escherichia coli* ST131 from Australia

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Abstract

Escherichia coli ST131 is a globally dispersed extraintestinal pathogenic *E. coli* lineage contributing significantly to hospital and community acquired urinary tract and bloodstream infections. Here we describe a detailed phylogenetic analysis of the whole genome sequences of 284 Australian ST131 *E. coli* isolates from diverse sources, including clinical, food and companion animals, wildlife and the environment. Our phylogeny and the results of single nucleotide polymorphism (SNP) analysis show the typical ST131 clade distribution with clades A, B and C clearly displayed, but no niche associations were observed. Indeed, interspecies relatedness was a feature of this study. Thirty-five isolates (29 of human and six of wild bird origin) from clade A (32 *fimH*41, 2 *fimH*89, 1 *fimH*141) were observed to differ by an average of 76 SNPs. Forty-five isolates from clade C1 from four sources formed a cluster with an average of 46 SNPs. Within this cluster, human sourced isolates differed by approximately 37 SNPs from isolates sourced from canines, approximately 50 SNPs from isolates from wild birds, and approximately 52 SNPs from isolates from wastewater. Many ST131 carried resistance genes to multiple antibiotic classes and while 41 (14%) contained the complete class one integron–integrase *intI1*, 128 (45%) isolates harboured a truncated *intI1* (462–1014 bp), highlighting the ongoing evolution of this element. The module *intI1–dfrA17–aadA5–qacEΔ1–sul1*–ORF–*chrA–padR*–IS*1600–mphR–mrx–mphA*, conferring resistance to trimethoprim, aminoglycosides, quaternary ammonium compounds, sulphonamides, chromate and macrolides, was the most common structure. Most (73%) Australian ST131 isolates carry at least one extended spectrum β-lactamase gene, typically $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$. Notably, dual *parC*-1aAB and *gyrA*-1AB fluoroquinolone resistant mutations, a unique feature of clade C ST131 isolates, were identified in some clade A isolates. The results of this study indicate that the the ST131 population in Australia carries diverse antimicrobial resistance genes and plasmid replicons and indicate cross-species movement of ST131 strains across diverse reservoirs.

DATA SUMMARY

The genome sequences of Orange Base Hospital (OBH) isolates (HOS77–HOS99) have been deposited in GenBank under BioProject PRJNA705804 with Sequence Read Archive (SRA) accession numbers SRR13843903 to SRR13843925.

The genome sequences of Melbourne Veterinary Collection (MVC) isolates have been deposited in GenBank under the following SRA accession numbers: SRR14629758, SRR14629798, SRR14629727, SRR14629683, SRR14629824, SRR14629814, SRR14629805, SRR14629641, SRR14629609 and SRR14629794.

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Abbreviations: AMR, antimicrobial resistance; APEC, avian pathogenic *E. coli*; ARGs, antibiotic resistance genes; EAEC, enteroaggregative *E. coli*; ESBL, extended spectrum β-lactam; ExPEC, extraintestinal pathogenic *E. coli*; MDR, multiple drug resistant; MVC, Melbourne Veterinary Collection; OBH, Orange Base Hospital; pMLST, plasmid multi-locus sequence typing; SAE, South Australia Environment; SAH, Sydney Adventist Hospital; SNP, single nucleotide polymorphism; UTIs, urinary tract infections; VAGs, virulence-associated genes; WGS, whole genome sequencing.

Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Eight supplementary data files are available with the online version of this article.

The genome sequences of Australian silver gull isolates (with 'CE' prefix and a single 1716 h isolate) have been deposited in GenBank under BioProject PRJNA630096 with SRA accession numbers SRR13834344 to SRR13834353.

The genome sequences of South Australia Environment (SAE) isolates are available at GenBank under BioProject PRJNA706036, SRA accession numbers SRR14763817 to SRR14763821.

For Sydney Adventist Hospital (SAH) isolates the genome sequenc of isolate SAH2009_36 has been published previously under SRA accession number SRX5100115. The genome sequences of all other isolates have been deposited in Genbank under BioProject PRJNA721525 with SRA accession numbers from SRR14229529 to SRR14229538.

Assemblies and annotated genomes for all Australian ST131 genomes used in this study are available with the online version of this article.

Supplementary material can be found in Figshare: https://figshare.com/s/8eb66db2f52bee86e5af.

Impact Statement

ST131 is a leading extraintestinal pathogenic *E. coli* (ExPEC) sequence type but the major clades A, B and C display different pathobiology. Clade C, particular C2, is considered the most clinically relevant as it is a frequent cause of urinary tract and bloodstream infections and resistant to multiple antibiotics, including extended spectrum β-lactams (ESBLs). However, its importance as a global ExPEC lineage is distorted because many studies focus on clinical ESBL-resistant ExPEC. Clade A ST131 are poorly studied but in terms of lethality are considered equivalent to clade C in a mouse sepsis model of infection, while clade B ST131 are often zoonotic pathogens with an established poultry link. Here we performed a phylogenetic analysis of 284 Australian isolates of ST131 irrespective of their drug resistance phenotype or source. We identified multiple clusters of highly related ST131 isolates representative of clades A, B and C especially among those from humans, companion animals and synanthropic birds. Evidence of transmission of closely related clade C2 isolates within and between different Australian hospitals was also observed. Notably, clade A isolates carrying $bla_{\text{CTX-M-27}}$ and dual *parC*-1aAB and *gyrA*-1AB fluoroquinolone resistant mutations, a feature previously considered unique to clade C isolates.

INTRODUCTION

Escherichia coli ST131, and emerging variants such as ST8196 [1], are significant contributors to hospital- and community-acquired urinary tract infections (UTIs) and bloodstream infections (BSIs) [1–4]. *E. coli* ST131 rose to prominence in 2008 and appeared simultaneously on three continents [5]. The ST131 lineage belongs to phylogroup B2 and most clinical isolates have an O25b:H4 serotype. *E. coli* ST131 with serotype O16:H5 is also well described, particularly in *fimH*41 isolates that carry the extended β-lactamase gene $bla_{\text{CTX-M-14}}$ [6].

The molecular phylogeny of *E. coli* ST131 describes three major sublineages, clades A, B and C, that are primarily delineated based on fimbrial adhesion type 1 (*fimH*) alleles and the carriage of antibiotic resistance genes [7, 8]. Clade A (*fimH*41), the most divergent of the three clades, probably emerged in Southeast Asia in the 1880s [9]. Recently, clade A isolates have been recovered from wastewater [10] that displayed resistance to drugs used to treat UTIs (fluoroquinolones, gentamicin, sulfamethoxazole-trimethoprim) [11–13]. In terms of lethality, clade A isolates with serotype O16:H5 are on a par with O25b:H4 in a mouse sepsis model of infection [6]. Australian isolates of ST131 with serotype O16:H5 are linked with men and reproductive-age women suffering with pyelonephritis [14].

Clade B probably emerged in the mid-1900s [9, 15] and carries the most diverse collection of fimbrial adhesion alleles (*fimH*22, *H*35, *H*27, *H*31 and *H*94) with the *fimH*22 allele predominating in about 65% of sequenced genomes [9, 16, 17]. ST131 clade B isolates are potentially zoonotic as isolates causing human disease have been linked with production of food animals [18], particularly poultry [19, 20]. Clade B isolates often carry ColV plasmids [18, 19], which are linked with the ability of avian pathogenic *E. coli* (APEC) to cause colibacillosis in poultry [21, 22], and contribute to pathogenesis in different animal models [23–25]. ColV plasmids carry an important arsenal of virulence-associated genes [22] and increasingly carry antibiotic resistance genes [26, 27]. ColV plasmid carriage has been linked to the ability of commensal *E. coli* to cause urosepsis [26], and novel *E. coli* sequence types such as ST301 [28] that have caused recent and severe outbreaks of haemolytic uremic syndrome in Europe and neonatal meningitis [23]. Of particular concern is a recent report of multiple drug resistant (MDR), *mcr*-carrying *E. coli* ST131 *fimH*22 (clade B) from poultry with colibacillosis in Brazil, the world's largest exporter of poultry products [29].

The results of several studies indicate that ST131 clade C split from clade B in North America between 1985 and 1994 [9, 15, 16, 30] and comprises three subclades: C0, C1 and C2 [31]. Clade C0 was established before clinical use of fluoroquinolones became widespread in human and veterinary medicine in the 1980s and is considered ancestral [16]. Clades C1 and C2 (almost exclusively *fimH*30) are globally dominant fluoroquinolone-resistant sublineages that carry dual mutations in chromosomal *gyrA* and *parC* genes. Clade C isolates include sublineage C1, defined as H30R and sublineage C2, referred to as H30Rx, which frequently carries the ESBL gene $bla_{\text{CTX-M-15}}$ that encodes resistance to the extended-spectrum cephalosporins used frequently in clinical medicine. Subpopulations of clade C isolates carry $bla_{\text{CTX-M-27}}$ or $bla_{\text{CTX-M-14,}}$ but their frequency is considerably less that those

that carry $bla_{CTX\text{-}M\text{-}15}$ [17]. It is posited that the widespread use of fluoroquinolones and extended-spectrum cephalosporins for the past 30 years provided a selection pressure that drove the global expansion of clades C1/*H*30R and C2/*H*30Rx [9]. ST131 clade C variants carrying genes encoding resistance to carbapenem antibiotics used to treat infections resistant to cephalosporins have also been described [32, 33]. Specific IncF-plasmid lineages have been linked to the different clade C lineages with F1:A2:B20 plasmids predominating in clade C1 and F2:A1:B− in clade C2 [31]. While F1:A2:B20 and F2:A1:B− plasmids are associated with CTX-M carriage and dissemination [34, 35], F29:A−:B10 plasmids, such as pUTI89, are linked to extraintestinal pathogenic *E. coli* (ExPEC) virulence [36].

E. coli ST131 is a significant contributor to UTIs and sepsis in Australia and elsewhere. The COmmunity Onset ESBL and AmpC *E. coli* Study (COOEE Study) identified ST131 to comprise about 8% of community-onset *E. coli* infections in Australia and New Zealand [37, 38]. In a large study of urinary tract isolates from reproductive age women (15–45 years of age) from the central west region of NSW, ST131 dominated isolates from patients with pyelonephritis and most also displayed resistance to fluoroquinolones. Resistance to fluoroquinolones was also a feature of ST131 isolates from patients with cystitis [14]. The results of a recent whole genome sequencing study of 67 trimethoprim-resistant ExPEC from patients with UTIs from a major regional hospital in New South Wales, showed that ST131 represented 28% of isolates [39]. Only one ST131 isolate carried a H27 *fimH* allele (clade B) with the remaining isolates carrying a H30 allele (clade C), the majority of which were ESBL $bla_{CTX\text{-}M\text{-}15}$-associated H30Rx (clade C2). Another Australian study that assessed 81 *E. coli* isolated from patients with bloodstream infections at Concord Hospital Sydney, identified 15 ST131, most of which were community acquired and MDR [36, 40].

E. coli ST131 has been isolated from pigs [18], poultry [19] companion animals [41] and wild birds [42] in Australia, but whole genome sequencing (WGS) has not been performed on a diverse Australian collection – from human, animal, and environmental sources – to examine diversity or interspecies transmission. Here we characterised the genomes of 284 ST131 isolates of Australian origin sourced from humans, companion animals, wastewater and urban wildlife to garner insights into the antibiotic resistance genes (ARGs) and virulence-associated genes (VAGs) they carry. A detailed phylogenetic and pangenome analysis was also undertaken.

METHODS

Genome sequences

This study used both published (n=226) and unpublished (n=58) *E. coli* ST131 genomes. For previously published sequences, EnteroBase (accessed 08.04.2020) was used to screen for all ST131 genomes isolated in Australia with year of isolation available in the associated metadata. All Enterobase sourced isolates have their sequence reads archive (SRA) accession numbers as isolate names. Short-read sequences of these genomes were downloaded using parallel-fastq-dump (github.com/rvalieris/parallel-fastq-dump). All genomes used in this study were assembled using shovill v1.0.4 (github.com/tseemann/shovill) using the SPAdes option and minimal contig length of 200 bp. All genomes underwent and passed quality control using assembly-stats software (github.com/sanger-pathogens/assembly-stats). Assembly statistics for this collection are available in Data S1 (available in the online version of this article). For unpublished sequences, the isolate sampling and sequencing methods used are described below.

Sampling and WGS of Australian ST131 isolates

Sampling and WGS methods for the 58 ST131 isolates sequenced for this study can be viewed in Data S2, which details sampling and WGS methods for Melbourne Veterinary Collection (MVC) isolates, Orange Base Hospital (OBH) isolates, Australian silver gull isolates, South Australia Environmental (SAE) isolates, and Sydney Adventist Hospital (SAH) isolates.

Phylogeny and SNP analyses

Construction of a maximum-likelihood SNP-based phylogenetic tree and calculation of pairwise SNP distances were executed using the snplord pipeline (github.com/maxlcummins/pipelord/tree/master/snplord), which combines snippy v4.3.6 (github.com/tseemann/snippy) with default settings, recombination filtering software Gubbins v2.3.4 (github.com/sanger-pathogens/gubbins) [43] with default settings, SNP-sites v2.4.1 (github.com/sanger-pathogens/snp-sites) with default settings, and FastTree v2.1.8 [44] using -gtr (generalised time-reversible model) and -nt options. For tree visualisation Interactive Tree of Life (IToL) v4 online based software [45] (itol.embl.de/) was used. Pairwise SNP distance heatmaps were visualised with R v4.0.2 using the packages ggplot2 (github.com/tidyverse/ggplot2) and ggtree [46] (github.com/YuLab-SMU/ggtree). Core genome phylogeny was constructed using IQtree2 [47] (iqtree.org/) with extended model selection and tree construction by best-fit model (-m MFP) and 1000 bootstrap replicates (-bb 1000) options and using a core genome alignment generated by Roary v3.13.0 [48] (sanger-pathogens.github.io/Roary/) using the fast core gene alignment with MAFFT (-e --mafft) option. FastBAPS grouping [49] was done using R package fastbaps V1.0.4, with default parameters. Third level FastBAPS was used, as it provided separation between ST131 C1 and C2 clades.

Gene screening

Gene screening was performed as described previously [39]. Briefly, serogroups were determined using SerotypeFinder v2.0 [50]. FimH typing was performed using FimTyper [50]. The ARIBA read-mapping tool with default settings was used to screen for VAGs, ARGs and plasmid replicons using the following reference databases: VirulenceFinder [51], ResFinder [51], PointFinder [52] and PlasmidFinder [52]. An additional custom database with antimicrobial resistance (AMR)-associated class 1 and 2 integrases, and additional ExPEC-associated VAGs was also utilised and can be accessed at https://githubcom/CJREID/custom_DBs. ARIBA data was processed using a bespoke script

accessible at https://github.com/maxlcummins/ARIBAlord and visualised in R v4.0.2 using package ComplexHeatmap [53] ggplot2 (github.com/tidyverse/ggplot2) and ggtree [46]. Plasmid multi-locus sequence typing (pMLST) was performed using pMLST v2.0 [52].

Presence of class 1 integrons was determined using BLAST+ (V2.8.1). Contigs harbouring class 1 integrons were further analysed in SnapGene (V4.1.9) (snapgene.com) and an integron map was drawn using graphics editing software Krita (V4.3.0) (krita.org). Similarly, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ genomic context maps were also analysed in SnapGene and drawn in Krita.

Plasmid map visualizations

A BLASTn-based map of pAA-ST131 and other ST131 *fimH*27 *E. coli* as well as pAPEC-O2-ColV were reconstructed using CGView Server (cgview.ca) [54]. pUTI89 plasmid mapping was done using BLAST based software ABRicate (github.com/tseemann/abricate) to screen for the plasmid sequence and the map was constructed using an R script available at (https://github.com/maxlcummins/plasmid_mapR) [21]. Isolates with more than 90% pUTI89 plasmid sequence coverage and 90% nucleotide identity were flagged as harbouring a 'pUTI89 like' plasmid. Plasmids were visualized using CGView Server.

Pangenome analysis

For assembly annotation, gene clustering and genome wide association study, Prokka v1.14.6 (github.com/tseemann/prokka) [55] using --compliant --mincontiglen 200 --genus Escherichia --species coli --gcode 11 options, Roary (sangerpathogens.github.io/Roary/) [48] and Scoary v1.6.16 (github.com/AdmiralenOla/Scoary) [56] with 'phenotype' set as subclades were used, respectively. Phandango (jameshadfield.github.io/phandango/) [57] was used for pangenome visualisation.

RESULTS

Demography

This study added 58 ST131 isolates to a pool of 226 ST131 isolates originating from Australia, and all 284 isolates were analysed here. Isolates from this study were sourced from humans (*n*=33; 23 OBH isolates, 10 SAH isolates), synanthropic birds (gulls) (*n*=10, CE isolates), canines (dogs) (*n*=10, MVC isolates), and wastewater (*n*=5, SAE isolates). The remaining 226 genomes were acquired from EnteroBase and were derived from human (*n*=183), urban and wild birds (gulls *n*=36, doves *n*=3, penguin *n*=1; total *n*=40), and poultry, porcine, canine (*n*=1 each). All isolates, bar the reference genome (EC958), originated from Australia with isolation dates ranging from 2005 to 2019, though the majority were acquired in 2017 (43%, *n*=122). All associated metadata is available in Data S3.

Phylogeny

To determine the evolutionary relationships within this ST131 collection, a maximum-likelihood phylogenetic tree was constructed using the complete genome of *E. coli* strain EC958 (GenBank accession number NZ_HG941718) as a reference. The maximum-likelihood tree was reconstructed using 8200 SNPs from an alignment of 91.2% to the reference genome (4.79 Mbp). The phylogenetic tree clearly shows the typical ST131 clade distribution with clades A, B and C prominently displayed. Subclades C1 and C2 are also distinguishable (Fig. 1). A C0 subclade was assigned based on the presence of the *fimH*30 allele [15] and the absence of *gyrA*-1AB and *parC*-1aAB SNPs [2]. The predominant serotype across clades B and C was O25b:H4 (*n*=218, 97%), while in clade A isolates O16:H5 was dominant (*n*=51, 86%).

Recombination filtering reduced the number of SNPs from 8200 to 2377, indicating that 71% of SNPs were introduced by recombination. Phylogenetic analysis utilizing SNPs without recombination filtering provided a similar topology and clade structure (Data S4). In both instances, there was no obvious host-based clustering, with human sourced isolates making up more than 70% (70% clade B – 81% clade C2) of all clades except C0 (100%, *n*=4). No gull sourced isolates were present in clades B and C0. Virotyping is another method used to characterise *E. coli* ST131 [58]. The method is based on the carriage of a particular set of virulence genes (*afa* FM955459, *iroN*, *ibeA*, *sat*). On the basis of this classification, virotype C (*afa* FM955459−, *iroN*−, *ibeA*−, *sat*+) (71%, *n*=201) was the most prevalent followed by virotype A (*afa* FM955459+, *iroN*−, *ibeA*−, *sat*+/−) (15%, *n*=42), virotype D (*afa* FM955459−, *iroN*+/−, *ibeA*+, *sat*+/−) (6%, *n*=16) and virotype B (*afa* FM955459−, *iroN*+, *ibeA*−, *sat*+/−) (1%, *n*=4). A total of 21 isolates (7%) could not be classified using this classification scheme. Virotypes demonstrated some cohesion with phylogeny. For example, all clade B isolates were virotype D and this virotype was restricted to these isolates, virotype B is only observed in clade C, and virotype C was most associated with clade C1 isolates.

The total pangenome of 284 Australian ST131 isolates identified 15050 genes (3453 core genes [>=99%] 400 soft core genes [>=95%], 1564 shell genes [>=15%] and 9633 cloud genes [<15%], with 3558 singletons). A gene presence/absence matrix and a genome wide association (GWA) analysis can be viewed in Data S5.

Pairwise SNP distance comparisons

Pairwise SNP distance heatmaps were produced to better illustrate the unique differences between A, B, and C clades. Three types of heatmaps were constructed, one without recombination filtering, one with recombination filtering, and one covering SNPs due to recombination only (Data S6). The comparisons of pairwise SNP distances between recombination filtered and unfiltered heatmaps clearly show that differences between and within clades is largely due to recombination, with the number of SNPs reduced approximately 25 times post-filtering (Table 1). On the basis of this observation

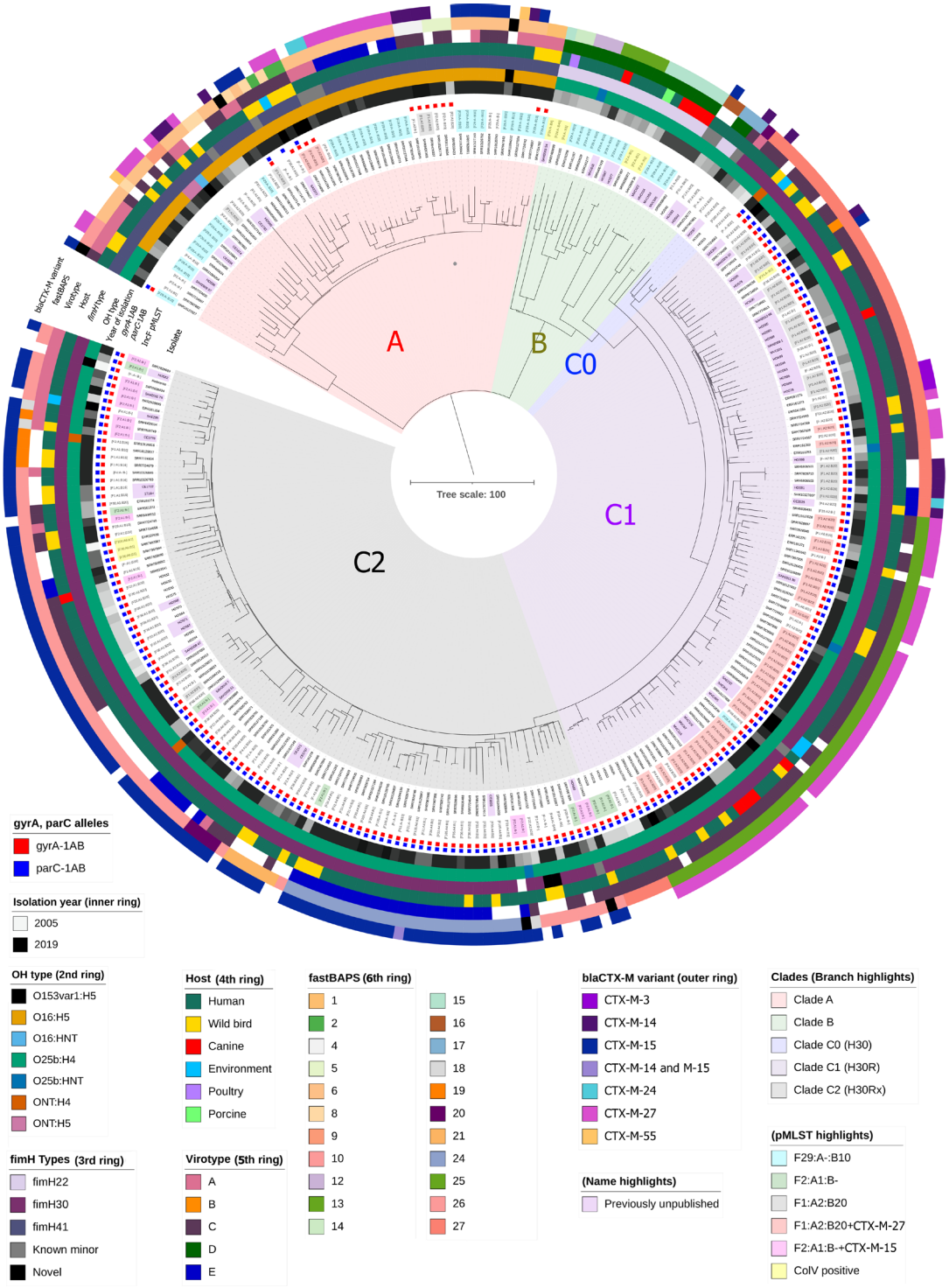

Fig. 1. Maximum-likelihood SNP-based phylogenetic tree of Australian ST131. Inferred using FastTree2, EC958 as a reference, and recombination filtering. Red and blue squares next to labels, denote presence of the characteristic fluoroquinolone resistance SNPs: *gyrA*-1AB (red) and *parC*-1aAB (blue). Coloured rings represent isolation dates (inner ring; colour gradient from light [2005] to dark [2019], serotype (second ring from centre), fimH type of isolate (third ring), host (fourth ring), virotype (fifth ring), fastBAPS groups (sixth ring) and bla_{CTX-M} variant (outer ring). Sections of the tree are coloured according to subclade (red=A, green=B, blue=C0, violet=C1 and black=C2). Inner tip label denotes isolate name, with names highlighted in purple representing previously unpublished genomes. Outer tip labels show which IncF plasmids are present in each isolate, with pMLSTs important for *E. coli* ST131 evolution highlighted. Tree scale bars represent the number of substitutions per site of alignment.

Table 1. Average SNP count difference between five clades. Numbers are average of recombination filtered/unfiltered

	Clade A				
Clade A	32/415	**Clade B**			
Clade B	136/3531	37/558	**Clade C0**		
Clade C0	125/3639	65/1670	24/420	**Clade C1**	
Clade C1	128/3548	70/1534	35/249	22/33	**Clade C2**
Clade C2	131/3560	73/1545	37/283	35/87	23/104

the heatmap of recombination only SNPs was constructed, and it was observed that within clades, clade C isolates had a low number of recombination only SNPs with an average of 58 (range: 0–1487), while clade B isolates exhibited higher recombination SNP variation with an average of 485 (0–1324) SNPs. Clade A had an average recombination SNP count of 383 (0–1505), however 35 out of the 59 isolates displayed a low recombination SNP count 23 (0–205) on average.

Closely related clade clusters

Our SNP analyses identified three clusters of closely related isolates from three clades - clade A, C1 and C2. It is important to identify potential for interspecies transmission and transfer from a public health perspective [59]. Thus, each closely related cluster was investigated further with additional SNP analyses performed using clade specific references and recombination filtering to provide greater SNP resolution.

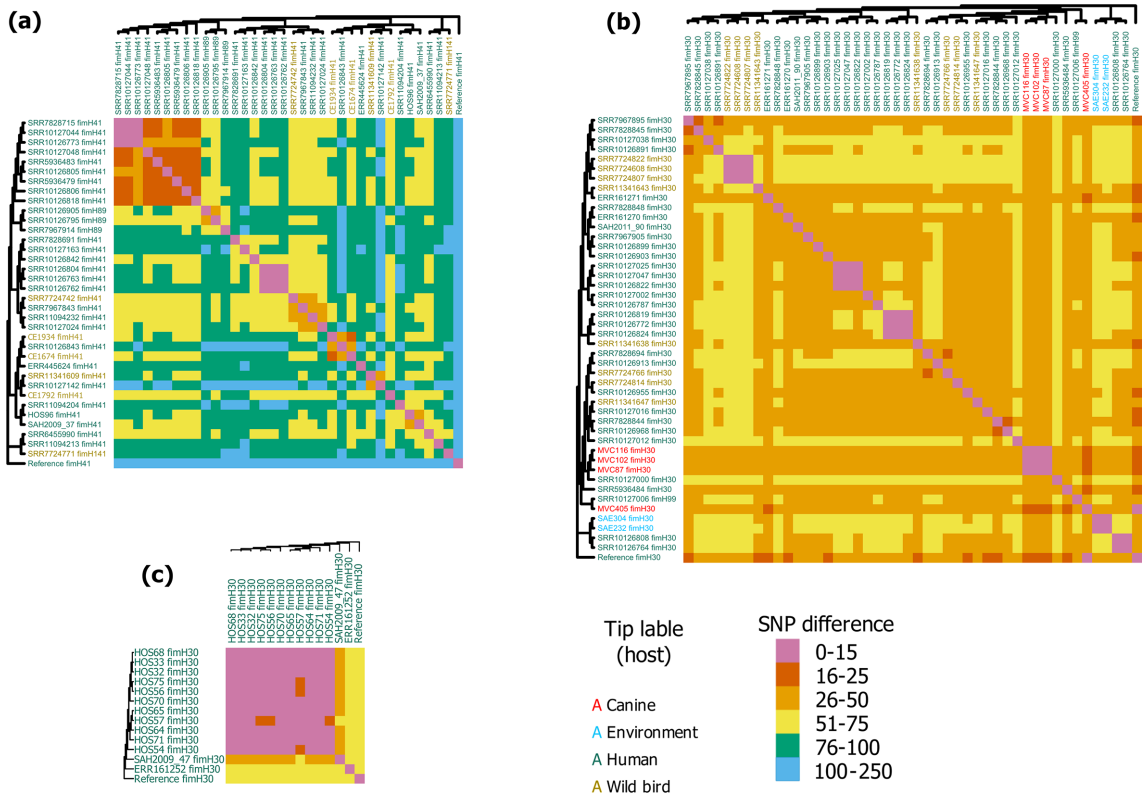

Fig. 2. Closely related clade clusters. (a) Heatmap illustrating pairwise SNP distances for 35 isolates in clade A. Both axes have a maximum-likelihood phylogenetic tree presented as a cladogram, constructed using these 35 isolates and clade A reference strain SE15 (ASM1048v1) (98% genome coverage; 4.73 Mbp; 970 SNPs total). (b) Heatmap illustrating pairwise SNP distances for 45 isolates from clade C1. Both axes have a maximum-likelihood phylogenetic tree presented as a cladogram, constructed using these 45 isolates and clade C1 reference strain 81 009 (ASM215724v1) (97% genome coverage; 4.98 Mbp; 711 SNPs total). (c) Heatmap showing SNP distances of 13 closely related hospital isolates from subclade C2. Both axes have a maximum-likelihood phylogenetic tree presented as a cladogram, constructed using only these 13 isolates, with EC958 as the reference strain (96% genome coverage; 5.02 Mbp; 133 SNPs total). All trees were constructed using recombination-filtered SNP alignments.

Thirty-five isolates from clade A (32 *fimH*41, 2 *fimH*89, 1 *fimH*141) were observed to differ by an average of 76 SNPs (Fig. 2a). These isolates originated from 29 human and six synanthropic bird samples. Isolates from synanthropic bird samples averaged 77 SNPs from human sourced isolates, with the smallest SNP count occurring between CE1674 (silver gull) with human sourced SRR10126843 at 45 SNPs. The average SNP distance between human sourced isolates was 76, however the cluster also contained nine human sourced isolates (two from 2015, seven from 2017) that differed by an average of 22 SNPs (0–31). The average SNP distance between synanthropic bird sourced isolates was 69 (23-88).

A set of 45 isolates from clade C1 from four sources (canine, wastewater, human, synanthropic bird) formed a cluster with an average of 46 (0–70) SNPs (Fig. 2b). The most closely related non-human sourced isolates to human-sourced isolates originated from canines (average 37 SNPs), followed by synanthropic birds (average 50 SNPs), and then wastewater (average 52 SNPs). The cross-host isolates with the lowest SNP counts were SRR7724766 (bird) and SRR7828694 (human) at 24 SNPs, SRR11341638 (bird) and MVC405 (canine) at 27 SNPs, and MVC405 (canine) and two human sourced isolates SRR5936484 and ERR161271 at 23 and 24 SNPs, respectively. Across human sourced isolates the average SNP count was 47 (3–68).

The third closely related cluster consisted of 13 isolates originating from three geographically distant hospitals – Orange Base Hospital (rural NSW), Sydney Adventist Hospital (urban NSW), and Princess Alexandra Hospital Brisbane (urban Queensland) – with an average SNP count of 19 (0–69) (Fig. 2c). All Orange Base Hospital isolates ('HOS' isolates) were exceptionally close at an average of 6 (0–19) SNPs despite being isolated from different patients and pathologies (seven patients with cystitis and four patients with pyelonephritis). Additionally, there were average SNP counts of 44 (41–54) SNPs from Sydney Adventist Hospital isolate (SAH2009_47) and 59 (55–69) SNPs from Princess Alexandra Hospital isolate (ERR161252).

Gene screening

This collection of 284 isolates was screened for the presence of VAGs, ARGs, plasmid replicons and IncF pMLSTs (Fig. 3, Data S3). Details are provided in the subsequent subsections.

AMR

A total of 44 ARGs were found in the Australian ST131 isolates (Fig. 3; Supplementary Data 3). Clade B isolates carried the fewest ARGs (Fig. 4a), with the average isolate possessing genes conferring resistance to only one antibiotic class (Fig. 4b) while the members of clade C2 were the most resistant, carrying on average ARGs conferring resistance to seven antibiotic classes. Human and gull sourced isolates presented similar numbers of ARGs carried, while canine sourced isolates had comparably lower carriage (Fig. 4c, d).

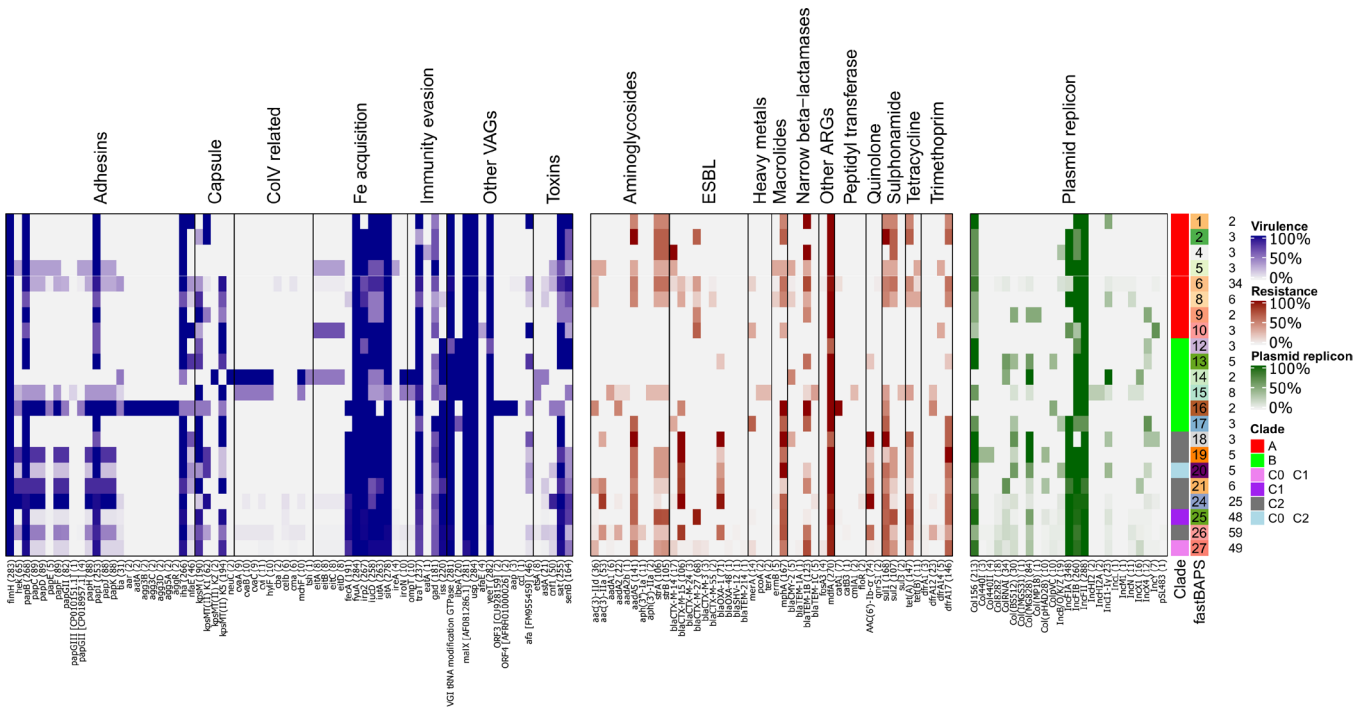

Fig. 3. ARG, VAG and IncF pMLST abundances in Australian ST131 population. Heatmap based on clustering by fastBAPS (fastBAPS groups consisting of singular isolates omitted). Relative gene abundance presented as blue (VAGs), red (ARGs) and green (plasmid replicons) colour gradients ranging from 0% (no colour) to 100% (most opaque). Total numbers of isolates carrying a gene are shown in parentheses after the gene name.

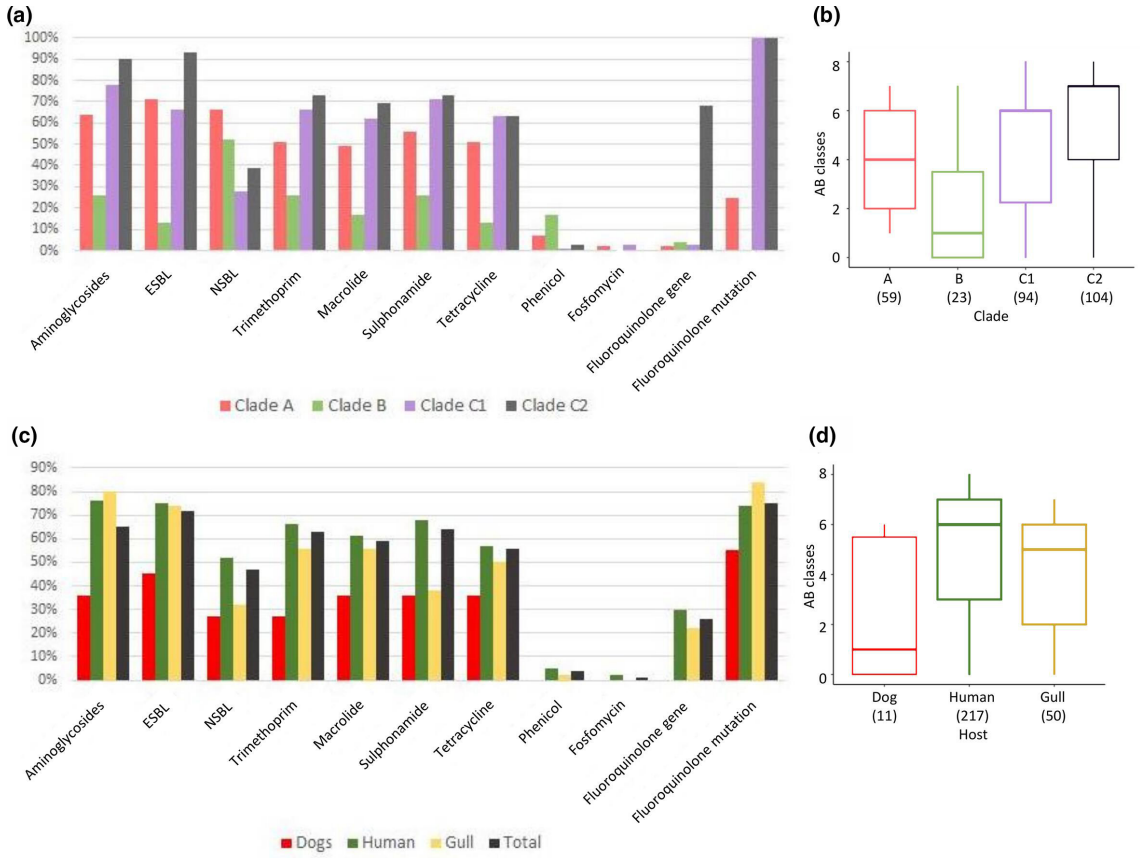

Fig. 4. Percentage of Australian ST131 isolates carrying ARGs conferring resistance to multiple antibiotics. (a) Percentage of isolates carrying one or more ARGs conferring resistance to various antibiotic classes across clades. Clade C0 isolates omitted due to the limited number (*n*=4). NSBL, narrow spectrum *β*-lactamase. (b) Box and whisker plot illustrating distribution of clade isolates carrying ARGs conferring resistance to antibiotic (AB) classes (c) Percentage of isolates carrying more than one ARG conferring resistance to various antibiotic class across hosts and in total. Environmental (*n*=5), porcine (*n*=1) and poultry (*n*=1) isolates omitted due to the limited numbers. Fluroquinolone mutation refers to the presence of *parC* and *gyrA* mutations. (d) Box and whisker plot illustrating distribution of host isolates carrying ARGs conferring resistance to AB classes. The presence of one or more genes counted as conferring resistance. Numbers in brackets are numbers of isolates.

At least one ESBL gene was present in 73% (*n*=206) of all isolates. Of the ten ESBL genes identified, $bla_{\text{CTX-M-15}}$ was most prevalent overall (*n*=106) followed by $bla_{\text{CTX-M-27}}$ (*n*=68). $bla_{\text{CTX-M-27}}$ was only present in clades A and C1, while $bla_{\text{CTX-M-15}}$ was most prominent in C2. Notably, a slightly higher percentage of clade A isolates (71%) carried ESBLs than did clade C1 isolates (65%).

Clade C1 isolates carrying $bla_{\text{CTX-M-27}}$ (C1-M27) reportedly carry a specific phage-like region- M27PP1 [60]. We screened for the presence of M27PP1 (>98% identity and >98% coverage) and found it in 49 isolates (17%) (Data S7), including three C1 isolates and one C2 isolate that do not possess $bla_{\text{CTX-M-27}}$ and 45 (92%) C1 isolates carrying $bla_{\text{CTX-M-27}}$. No clade A isolate, $bla_{\text{CTX-M-27}}$ positive or negative, harboured M27PP1. The most common narrow-spectrum *β*-lactamase (NSBL) gene was $bla_{\text{TEM-1B}}$ across all hosts (*n*=123). $bla_{\text{OXA-1}}$ was heavily associated with clade C2 isolates (99%, *n*=70 out of 71).

Genes conferring resistance to aminoglycosides, macrolides, sulphonamide, trimethoprim and tetracyclines were also prevalent among Australian ST131 isolates. Of the ten aminoglycoside resistance genes identified, the most common were *aadA5* (50%, *n*=141) followed by *strA* (37%, *n*=106) and *strB* (37%, *n*=105). Macrolide resistance gene *mphA* was found in 59% of isolates (*n*=167), while *ermB* was only identified in two gull and three human sourced isolates. Sulphonamide resistance gene *sul1* was also identified in 59% of isolates (*n*=168) while *sul2* was identified in 38% (*n*=107). *sul*3 was only seen in the single porcine sourced isolate and one human sourced isolate, and these two isolates differed by 10 SNPs, as previously described [18]. Trimethoprim resistance gene *dfrA17* was the most common of this class and was the only trimethoprim resistance gene identified in gull (56%, *n*=28), environmental (80%, *n*=4), and canine (27%, *n*=3) sourced isolates. While *dfrA17* was also the most abundant in human sourced isolates (51%, *n*=111), other trimethoprim resistance

genes were also identified in this cohort including *dfrA12* (10%, *n*=22), *dfrA14* (2%, *n*=5), *dfrA1* (1%, *n*=3), *dfrA7* (*n*=1). Tetracycline resistance gene *tetA* was identified in 52% of all isolates (*n*=147) while *tetB* was only present in 11 (4%) isolates, and only those originating from gulls and humans.

Conversely, genes conferring resistance to phenicol, fluroquinolone and fosfomycin were less prevalent in Australian ST131 isolates. These genes included phenicol resistance genes *catA1* (2%, *n*=7), *catB3* (*n*=1), *cmlA1* (1%, *n*=2) and *floR* (1%, *n*=2); and fosfomycin resistance gene *fosA3* (1%, *n*=4) and fluroquinolone resistance gene *qnrS1* (1%, *n*=2). The aminoglycoside gene with extended fluoroquinolone resistance *acc(6')-Ib-cr*, was almost exclusively found in clade C2 isolates (71 out of 74; 96%). Genes conferring resistance to phenicol and fluroquinolones occurred only in human and gull sourced isolates, and to fosfomycin only in human sourced isolates. Only one Australian ST131 isolate carried a carbapenem resistance gene ($bla_{\text{OXA-48}}$) and this isolate was of clade C1 and human sourced.

Regarding mutations conferring resistance to fluoroquinolone, 74% (*n*=212) of ST131 *E coli* isolates had *gyrA*-1AB mutations and 71% (*n*=202) had *parC*-1aAB mutations with 71% (*n*=201) isolates having both mutations. Clade C accounted for 198 isolates with double gene mutations, the remaining three belonged to clade A isolates. Some uncommon mutations were also identified, including *gyrA*-1AD (one clade A seagull sourced isolate), *parC* (E84K) (one clade B human sourced isolate) and *parC* (S57T) (one clade C human sourced isolate). Of these uncommon mutations, only *gyrA*-1AD has previously been shown to confer fluoroquinolone resistance [61].

Class 1 and 2 integrons and $bla_{\text{CTX-M-15/27}}$ genetic contexts

Class 1 integrons play a central role in AMR because they can capture and express diverse resistance genes and their presence is considered a reliable proxy for an MDR genotype [62]. In Australian ST131 isolates, full-length class 1 integron-integrase *intI1* was present in 41 isolates (14%), and a class 2 integron-integrase *intI2* was identified in one isolate. Notably, truncated *intI1* genes were also detected in 128 isolates (45%) with *ΔintI1* ranging from 462 to 1014 bp. These truncations serve as potential epidemiological markers to track MDR ST131 and the mobile elements they carry.

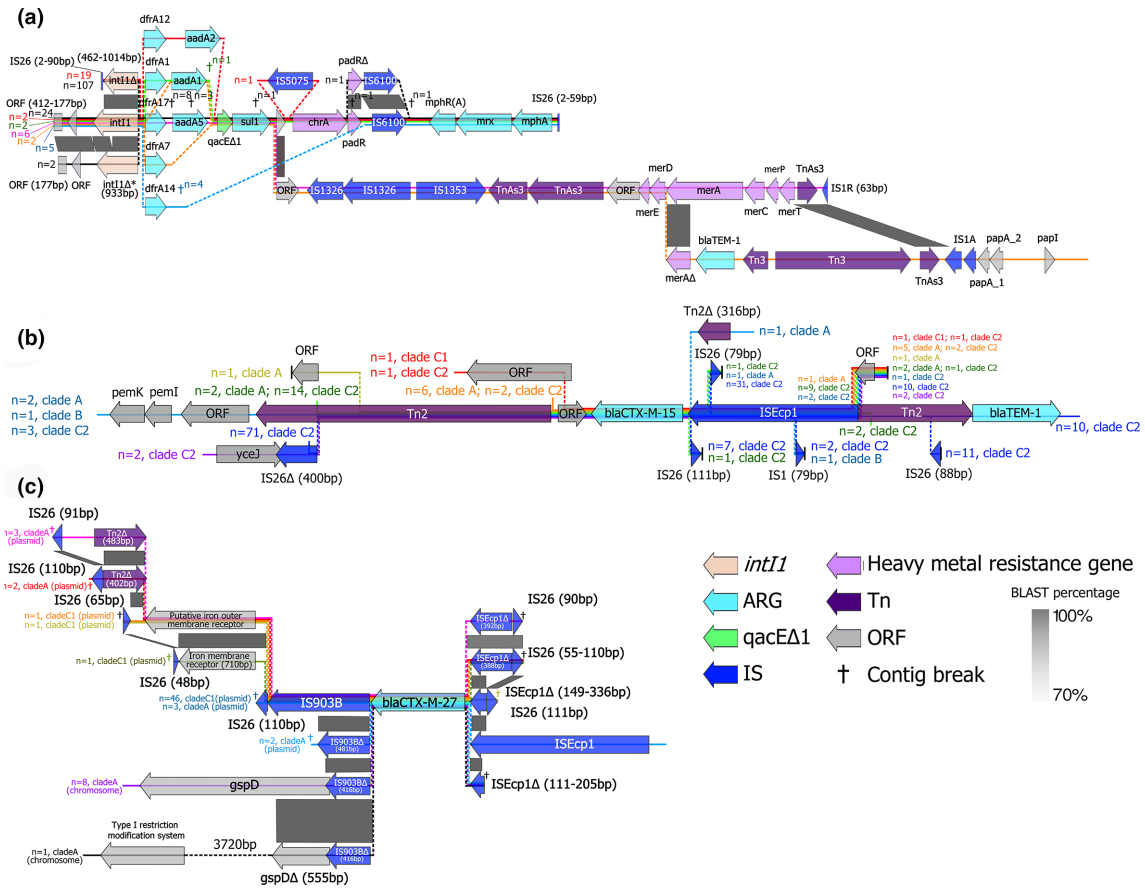

Fig. 5. Class 1 integron structures and $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$ genomic contexts. (a) Schematic map of class one integrons present in Australian ST131 isolates. (b) Schematic map of genomic context of $bla_{\text{CTX-M-27}}$. (c) Schematic map of genomic context of $bla_{\text{CTX-M-15}}$. In each panel, solid-coloured lines represent individual structures with dashed lines representing variations and connections between structures. Contig breaks denoted by '†'.

Six primary class 1 integron structures were observed (Fig. 5a), the most abundant being *intI1–dfA17* (trimethoprim resistance)–*aadA*5 (aminoglycoside resistance)–*qacEΔ1* (quaternary ammonium compounds resistance)–*sul1* (sulphonamide resistance)–ORF–*chrA* (chromate resistance)–*padR*–IS*6100*–*mphR*–*mrx*–*mphA* (macrolide resistance) found in 47% (*n*=101) of human, 50% (*n*=25) of seagull, 27% (*n*=3) of canine and 80% (*n*=4) of environment isolates (structure on the black line in Fig. 5a). Six integron structures carried the mercury resistance transposon Tn*As3* (structure on the pink line in Fig. 5a) found in clade B (*n*=1) and clade C1 (*n*=5) human sourced isolates and two structures (orange line) carried Tn*3* transposon with $bla_{\text{TEM-1B,}}$ found in one clade A and B human sourced isolates. Only one isolate (SRR10126957) contained a class 2 integron structure (*intI2–dfrA1–sat2–aadA1*) carried by a clade C2 human sourced isolate from 2017.

Regarding the genetic context of $bla_{\text{CTX-M-15/27}}$ genes, the majority of $bla_{\text{CTX-M-15}}$ genes were located adjacent to IS*Ecp1* (92%, *n*=97) and downstream of Tn*2* (91%, *n*=96) (Fig. 5b). Notably, 15 $bla_{\text{CTX-M-15}}$ genes were localised to the chromosome (in 2 clade A, and 13 clade C2 isolates). Concerning the genetic context of $bla_{\text{CTX-M-27}}$, in all examples the gene was situated adjacent to IS*Ecp1* and IS*903B* (Fig. 5b). Nine clade A isolates had this insertion within the chromosome, one of which was situated 4219 bp upstream from the type I restriction modification system gene and eight isolates had insertions near a general secretion pathway (*gsp*) operon. These insertion sites serve as epidemiological markers in future ST131 studies. The remaining 59 isolates had $bla_{\text{CTX-M-27}}$ situated on small contigs with IS*26* situated at contig breaks, and too few genes to ascertain their genetic context. Three clade A isolates carried an identical IS*Ecp1*-$bla_{\text{CTX-M-27}}$–IS*903B* structure seen in the majority of C1 isolates (Fig. 5c).

VAGs

A total of 72 VAGs were identified in the Australian ST131 isolates (Fig. 3, Data S3). Present in all isolates were ferric yersiniabactin uptake receptor *fyuA* and uropathogenic specific protein *usp*. Other key VAGs featured here were the Sit ferrous iron utilisation system gene *sitA* (98%, *n*=258), aerobactin genes *iutA* (93%, *n*=263) and *iucD* (91%, *n*=258), adhesin *iha* (94%, *n*=266), secreted autotransporter toxin *sat* (90%, *n*=255) and group two capsule gene *kpsMT-II* (91%, *n*=258). The *kpsMT-II* K5 allele was the most common across all Australian ST131 isolates (*n*=194) and dominated all clades and hosts except for clade C2 isolates, wherein 50% (*n*=52) carried the K1 allele while 42% (*n*=44) carried K5. Only two isolates carried *kpsMT-II* K2 (both clade B). The major components of the pyelonephritis-associated pili operon (*papBCDFGHIJK*) were present in 87 isolates (31%), however isolates contained varying degrees of *pap* gene representations, ranging from 94% of isolates containing *papB* (*n*=268) to 2% carrying *papE* (*n*=5). In terms of average total number of VAGs, clade C2 isolates had the highest (*n*=25) followed by clade B isolates (*n*=23), though clade B isolates contained several unique VAGs including those associated with enteroaggregative *E. coli* (EAEC) such as *agg3BCD, agg5A* and *aggR,* as well as those associated with neonatal meningitis-causing *E. coli* (NMEC) such as *ibeA* and *neuC*.

Plasmid replicons, IncF pMLSTs and virulence plasmid comparisons

A total of 13 plasmid incompatibility groups were identified in Australian ST131 isolates (Fig. 3, Data S3), the most abundant being IncFII (96%, *n*=273) followed by IncFIB (85%, *n*=242) and IncFIA (71%, 202). Other incompatibility groups detected were IncI1 (8%, *n*=23), IncB/O/K/Z (7%, *n*=19), IncX4 (4%, *n*=12), IncX1, IncN (each 4%, *n*=11), IncY (2%, *n*=7), IncHI2, IncHI2A (each 1%, *n*=2), IncL and IncM1 (each *n*=1).

Among the IncF plasmids a total of 67 pMLSTs were identified, the most common being F1:A2:B20 (25%, *n*=70), F29:A–:B10 (16%, *n*=44) and F2:A1:B– (8%, *n*=23). The dominant pMLST type in clades A and B was F29:A–:B10 (both 53%), F22:A1:B20 in clade C0 (75%), F1:A2:B20 in clade C1 (65%) and F2:A1:B– in clade C2 (19%). F1:A2:B20 and F2:A1:B- are well described and dominant F plasmids in ST131 clade C sublineages [31].

F29:A–:B10 is an important IncF pMLST found in several pandemic, but pan-susceptible, ExPEC lineages [63]. F29:A-:B10 is also known to be pMLST of the pUTI89 virulence plasmid [36]. F29:A-B10 was the dominant IncF pMLST in Australian clade A and B ST131 isolates. Using pUTI89 (NC_007941.1) as a reference we identified 46 isolates that potentially carry pUTI89-like plasmids (Fig. 6a). Notably 49% of clade A isolates and 48% of clade B isolates potentially carry a pUTI89-like plasmid, while conversely only 3% of any clade C isolates did. Notably, 13 isolates carrying pUTI89-like plasmids also carried $bla_{\text{CTX-M-27}}$, nine of which were localised to the chromosome (all clade A) and the genetic context for the remainder could not be determined due to small contig size. Similarly, 11 pUTI89-like plasmid carriers also harboured $bla_{\text{CTX-M-15}}$, five of which were chromosomal and the remainder at indeterminable locales. It was also noted that pUTI89-like cargo was associated with fewer plasmid replicons [average 0.7, (0–4) vs average 1.9 (0–6), Wilcoxon Test $P=9.6\times10^{-12}$] in ST131.

ColV plasmids are also associated with ExPEC virulence [24]. On the basis of the criteria of Liu *et al.* [19] ten ColV-positive isolates were identified (six in clade B, three in clade C2, one in clade C1), consisting of four types: *iuc* (aerobactin iron acquisition system) and *ets* (*E. coli* transport system) operon-positive, *eit* (ABC iron transport system)-negative (*n*=6); *ets*-positive, *iuc*- and *eit*-negative (*n*=2); *iuc*-positive, *ets*- and *eit*-negative (*n*=1); *iuc*- and *eit*-positive, *ets*-negative (*n*=1). Using pAPEC-O2-ColV (NC_007675) as a reference, all ten putative ColV plasmids contained *cvaABC* (colicin V), *iroB-CDEN* (salmochelin iron acquisition system) and *sitABCD* (ABC iron transport system) regions (Fig. 6b).

Two *fimH*27 isolates (clade B) were found harbouring aggregative adhesion fimbria (AAF) genes (*agg3BCD, agg5A*) known to be carried on virulence plasmid pAA, a defining feature of EAEC [64]. A hybrid ST131 ExPEC/EAEC lineage carrying pAA caused an outbreak in Denmark (1998 to 2000)

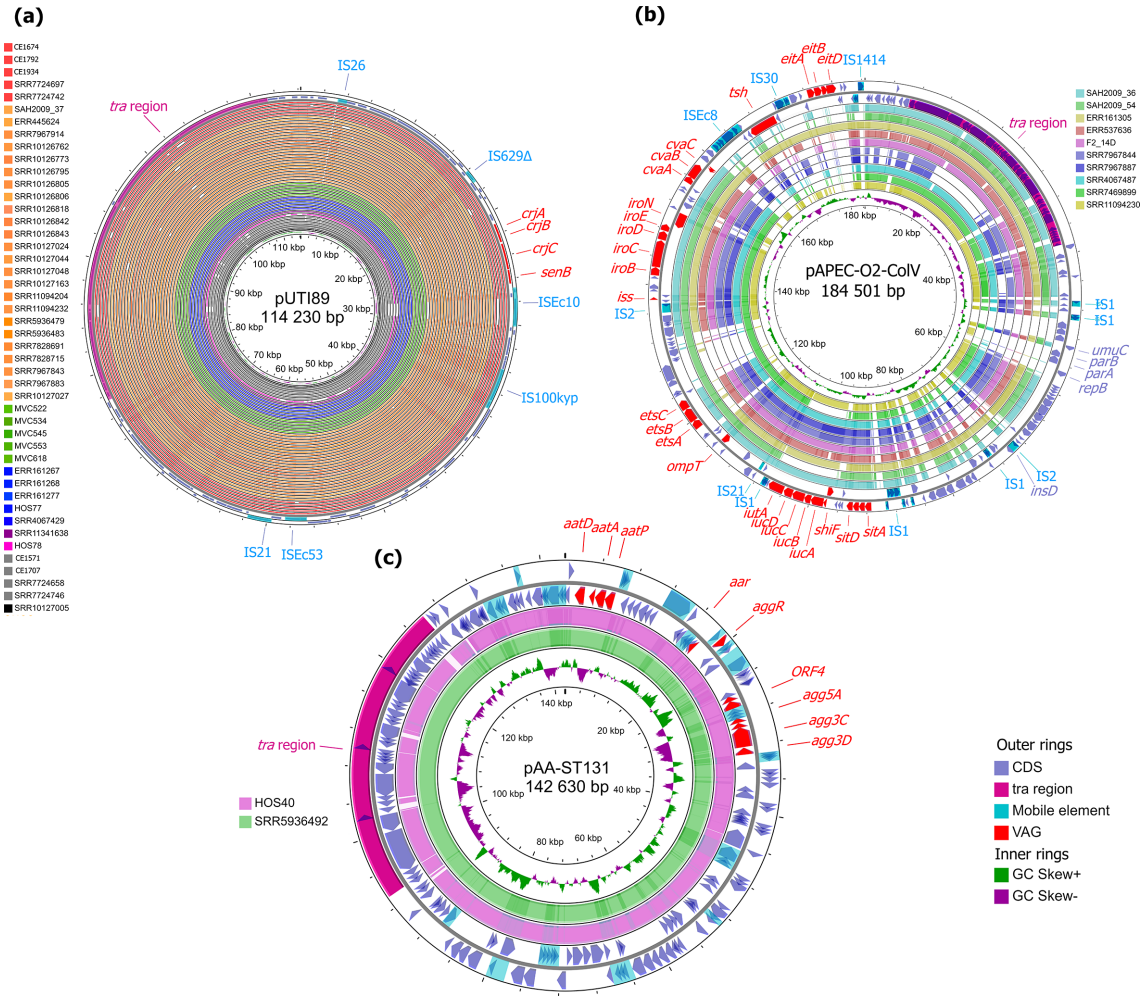

Fig. 6. BLAST comparisons of three virulence plasmids using CGview. (a) Comparisons of pUTI89-like plasmids present in 43 Australian ST131 isolates. Rings coloured by isolate source and clade: red=wild bird, clade A; orange=human, clade A; green=canine, clade B; blue=human, clade B; purple=wild bird, clade C1; pink=human, clade C1; grey=wild bird, clade C2; black=human, clade C2. (b) Comparisons of pAPEC-O2-ColV-like plasmids present in ten isolates (c) Comparisons of pAA-like plasmids present in two isolates. VAGs are coloured in red, mobile elements in blue, *tra* region in scarlet, inner circle show GC skew.

[65]. While a phylogenetic analysis demonstrated that the two Australian isolates were not closely related to any of the Denmark outbreak strains (Data S8), a BLASTn comparison of pAA-ST131 (KY706108; plasmid from a Danish outbreak strain) illustrated that they both potentially carry similar plasmids (Fig. 6c).

DISCUSSION

The *E. coli* ST131 lineage was first reported in Australia in 2010 in a study that collected *E. coli* samples from six microbiology laboratories between 2008 and 2009 and from a total of 15 ST131 isolates identified, four produced CTX-M-15 [66]. Here, approximately a decade later, we added 58 ST131 genomes to a total pool of 284 ST131 WGS of Australian origin sourced from humans, animals and the environment. Through genomic analyses several key observations on the Australian ST131 population were made including (1) no clear indication that ST131 clades are niche associated; (2) ESBL genes $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ dominated; (3) most clade A isolates carried ESBL genes (71%), some of which harboured chromosomal $bla_{\text{CTX-M-27}}$ and others carried dual *parC*-1aAB and *gyrA*-1AB mutations conferring ciprofloxacin resistance, and (4) there was a greater diversity and distribution of F plasmids pMLSTs across clades than previously noted.

Research has emerged indicating that ST131 clades are niche associated, specifically that clade C has adapted to human hosts [3], clade B is zoonotic and dominates meat, pigs [18, 67] and poultry [19], and that clade A is predominantly environmental [10]. Others claim genetic commonality and a broad distribution across ecological niches and reservoirs [68, 69]. While cautious to extrapolate from a dataset heavily skewed towards human sourced ST131 isolates (76%), our

results aligned more with the latter argument of no clear clade orientated niches. Each clade (A, B, C1 and C2) was comprised of approximately ¾ human sourced and approximately ¼ non-human sourced isolates and our phylogenetic analyses did not demonstrate any distinct clustering of these non-human isolates within any clade. Instead, the analysis demonstrated canine, porcine, poultry, synanthropic bird (silver gull) and wastewater sourced isolates dispersed among the human sourced, indicating cross-species transmission. Indeed, our SNP analyses identified clusters of highly related cross-species isolates, with the smallest SNP distance observed between human and silver gull sourced clade C1 isolates (24 SNPs). Clade C1 was found to be particularly clonal with an average of 22 core SNPs. Examples of small cross-species SNP counts in ST131 isolates have been described previously, including an Australian clade B porcine and clinical ST131 isolate separated by 22 SNPs [18] and a clade C1 cat urine (Australia) and human faecal (Spain) ST131 isolate separated by 66 SNPs [41].

When arguing genetic commonality, recombination should be addressed [70]. Recombination facilitates the integration of exogenous DNA, including MGEs, thereby promoting and accelerating adaptive diversification in bacteria [71–73]. Probably due to a versatility in infection sites, the rate of recombination is particularly high in ExPEC [71, 72], and as much as 77% of SNPs have been attributed to recombination in ExPEC ST131 [7]. This high percentage is congruent with our own 71% of SNPs due to recombination identified. Previous studies have shown that recombination filtering is required to elucidate true ST131 tree topology [16] and indeed in this study only through recombination filtering was the C0 clade made evident. Nevertheless, without recombination filtering, the topology of our phylogenetic tree remained largely unchanged with non-human sourced isolates distributed among human sourced isolates, and closely related cross-species clusters remained intact. This indicates that while recombination contributes considerably to ST131 diversity, it does not alter the apparent cross-species movement within clades.

The *E. coli* ST131 lineage is notorious for having played a central role in the worldwide increase of ESBL-producing *Enterobacteriaceae* which was driven by the capture of $bla_{\text{CTX-M}}$ genes from the environment [74]. Copious studies have touted the MDR profiles of ST131 isolates worldwide [2, 3, 75] and the Australian ST131 population appears no different. While only a single Australian ST131 isolate carried a carbapenemase and none carried colistin resistance genes, on average ST131 isolates from Australia carried genes resistant to five antibiotic classes, ranging from clade B isolates averaging resistance to one class, to C2 isolates averaging resistance to seven classes. C2 isolates carried more class one integrons, which are considered reliable indicators for a MDR genotype [62] although notably many of the integrase genes were truncated and therefore are unlikely to be detected with standard PCR primer sets. Overall, the most common structure carrying class 1 integron found in Australian ST131 isolates was *intI1–dfrA17–aadA5–qacEΔ1–sul1*–ORF–*chrA*–*padR*–IS*1600*–*mphR*–*mrx*–*mphA* conferring resistance to trimethoprim, aminoglycoside, quaternary ammonium compounds (disinfectant), sulphonamide, chromate (metal) and macrolides. Identical integrons have been previously observed in Australian clinical ST131 and ST200 isolates [39] as well as an ExPEC ST410 isolate from a canine in Portugal [76]. Clade C2 isolates were also strongly associated with $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$ and the acquired aminoglycoside gene with extended fluroquinolone resistance *acc(6′)–Ib–cr,* all of which are well documented in this clade globally [77–79]. In clade C2, class one integrons, $bla_{\text{CTX-M-15}}$ and other AMR genes are most commonly carried on F2:A1:B– plasmids [34, 80, 81]. Indeed, this pMLST is thought to have shaped the evolution of C2 [31]. Interestingly, while F2:A1:B– was the most common pMLST in the Australian cohort of clade C2, it was only present in 20 isolates (19%). The remaining isolates carried F plasmids of 35 different pMLSTs, thus the previously reported strong correlation between C2 and F2:A1:B– plasmids was not observed in this ST131 population.

ST131 clade C1 is also associated with ESBL production worldwide and most commonly harbours $bla_{\text{CTX-M-14}}$ [17]. However, a subpopulation of C1 isolates carrying $bla_{\text{CTX-M-27}}$ (C1-M27), providing greater ceftazidime resistance [82], was first identified in Japan in 2006 [60] and has since emerged globally and is being reported at increasing frequencies in both humans and animals [80, 83–86]. In Australia, we found C1 isolates most frequently carried $bla_{\text{CTX-M-27}}$ (52%), while only 6% carried $bla_{\text{CTX-M-14.}}$ Intriguingly, though typically reported as antibiotic sensitive [77], 71% of Australian clade A isolates carried at least one ESBL gene, of which $bla_{\text{CTX-M-27}}$ was most prevalent. While biassed selection criteria cannot be ruled out as a cause of the high percentage of ESBL resistant clade A isolates observed here, we did note that several clade A isolates had chromosomal $bla_{\text{CTX-M-27}}$ genes while others shared the same genetic context as C1-M27 isolates. Furthermore, three clade A isolates possessed the dual *parC*-1aAB *gyrA*-1AB mutations previously only reported in clade C. These two mutations not only confer resistance to fluroquinolone but also improve fitness in the absence of antibiotics [87] and it is generally acknowledged that the combination of fluroquinolone and ESBL resistance propelled the global expansion of clade C [7]. It is therefore alarming that clade A may be following suit and should be monitored closely, especially when population estimates have flagged clade A as the dominant clade in Australia [37], and unbiased sampling methodologies have also identified clade A as dominant in the community in PR China [88] and domestic wastewater in Canada [10].

In terms of virulence factors, ST131 isolates from Australia did not deviate from previous reports in that most (>90%) carried yersiniabactin receptor *fyuA*, uropathogen-specific protein *usp*, pathogenicity island marker *malx*, secreted autotransporter *sat,* aerobactin genes *iutA* and *iucD* and capsule *kpsM II* [6, 89]. Notably the *kpsM II* K2 allele has previously been reported to be strongly associated with clade A [6], however in this collection K5 was the most common allele in clade A and C1, K1 in C2 and the only two examples of K2 alleles originated from clade B.

Given that ColV and pUTI89-like plasmids are associated with ExPEC virulence [90] we performed plasmid mapping and ascertained that ST131 ColV plasmid carriage is low (n=10) in this Australian collection. ColV plasmids are a defining feature of APEC [19, 21] and the low carriage here may be due to the collection being largely human sourced, though the single poultry isolate did carry a ColV-like plasmid. Conversely, pUTI89-like cargo, which is linked to bacterial invasion [90], was present in approximately half of all clade A and B isolates, though only present in 3% of clade C isolates. The presence of pUTI89 has previously been reported to reduce the number of other plasmids in the ST95 ExPEC lineage and has not been reported as carrying ARGs [63]. Here we found that the presence of pUTI89-like cargo was also associated with fewer replicons in ST131. Notably, we identified several isolates harbouring pUTI89-like plasmids that also carried $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$, however, where genetic context could be ascertained, these ESBL genes were always located on the chromosome.

Lastly, a subclade of H27 ST131 isolates have been recently described as hybrid EAEC/ExPEC strains due to the acquisition of a pAA plasmid carrying genes that code for aggregative adhesion fimbria which are central to EAEC pathogenesis and prolific biofilm formation [65, 91]. A hybrid H27 sublineage has reportedly caused an outbreak of UTIs and bacteraemia in Denmark [65]. Here we found that the only two H27 ST131 isolates in this collection (two human sourced clade B isolates) carried a pAA-like plasmid, though these isolates were not closely related to the Danish outbreak strains.

Limitations

In addition to the aforementioned skew towards human sourced ST131 isolates, this study also had the limitation of Enterobase genome selection by ST, Australian origin and year of isolation only. It is unknown whether these ST131 isolates had associated selection biases, such as ESBL screening or pathologies. Additionally, given that 43% of Enterobase-extracted isolates were isolated in 2017, no meaningful temporal studies could be conducted.

CONCLUDING STATEMENT

In conclusion, our findings demonstrate that the ST131 population in Australia carry genes conferring resistances to multiple antibiotic classes, carry similar virulence cargo and indicate no niche associations. This highlights the importance of shifting emphasis from solely clinically focused sampling as it seems likely that strains can move across ecological niches. A better understanding of ST131 and ExPEC reservoirs could lead to the development of preventative measures regarding the spread of infection and antimicrobial resistance.

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Author contributions
D.L.: formal analysis, investigation, visualisation, writing – original draft. E.R.W.: data curation, formal analysis. P.E.: sequencing and analysis of canine ST131. E.D. and B.D.; sampling and isolation of environmental ST131 isolates. D.B. and I.G.C.: sequencing of environmental ST131 genomes. P.R.C. and J.M.: sequencing and analysis of hospital ST131 isolates. T.K.: isolation and investigation of hospital ST131 isolates. G.F.B., M.S.M. and R.N.B.: isolation and provision of canine isolates. N.H. and N.M.: isolation and provision of hospital ST131 isolates. M.D.: isolation and microbiological and molecular biological analysis of ST131 isolates from gulls. V.M.J.: conceptualisation, formal analysis, supervision, visualisation, writing – original draft, review and editing and S.P.D.: conceptualisation, supervision, funding acquisition, project administration, writing – original draft, review and editing.

Conflicts of interest
The authors declare that the funding bodies had no direct influence on the study design, collection, analysis/interpretation of data, or in the writing of this report.

Ethical statement
Acquisition of Orange Base Hospital (OBH) isolates was approved by Charles Sturt University and Sydney West Area Health Service research ethics committees. Since clinical information for patients with UTIs was provided anonymously by clinicians, patient consent was not required. No animal ethics was required in the acquisition of the Melbourne Veterinary Collection (MVC) isolates as all isolates were obtained from patients as part of veterinary clinical diagnostic procedures.

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