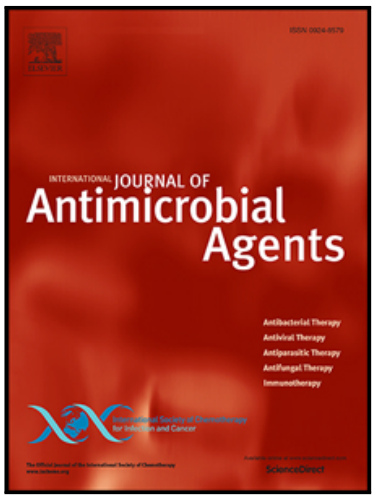

Genomic analysis of multiple drug resistant Escherichia coli ST58 causing urosepsis

Jessica McKinnon , Piklu Roy Chowdhury , Steven P. Djordjevic

PII: S0924-8579(18)30185-7
DOI: 10.1016/j.ijantimicag.2018.06.017
Reference: ANTAGE 5475

To appear in: *International Journal of Antimicrobial Agents*

Received date: 3 February 2018
Revised date: 18 June 2018
Accepted date: 23 June 2018

Please cite this article as: Jessica McKinnon , Piklu Roy Chowdhury , Steven P. Djordjevic , Genomic analysis of multiple drug resistant Escherichia coli ST58 causing urosepsis, *International Journal of Antimicrobial Agents* (2018), doi: 10.1016/j.ijantimicag.2018.06.017

Highlights:

- First report of uropathogenic *Escherchia coli* ST58 (strain 2009-52) from a patient with urosepsis
- Illumina and Single Molecule, Real-Time (SMRT) sequencing used to characterise strain 2009-52
- Strain 2009-52 carries IncF plasmid pSDJ2009-52F with antimicrobial resistance and virulence genes
- pSDJ2009-52F carries an atypical class 1 integron and Tn*6029* within a novel Tn*2610*-like transposon
- pSDJ2009-52F is closely related to pSF-088-1 from *E. coli* ST95 from a patient with blood sepsis

Genomic analysis of multiple drug resistant *Escherichia coli* ST58 causing urosepsis

Jessica McKinnon[1§], Piklu Roy Chowdhury[1,2,§] and Steven P Djordjevic[1,*]

[1]*ithree Institute, University of Technology Sydney, PO Box 123, Broadway, Sydney, NSW, Australia, 2007*
[2]*Department of Primary Industries, Elizabeth Macarthur Agriculture Institute, PMB 4008, Camden, NSW 2567, Australia*

Keywords: *E. coli* ST58, ExPEC; virulence plasmid; complex resistance region; Tn*6029*, Tn*2610*

§ Authors have contributed equally.

***Author for correspondence**

Steven P. Djordjevic

ithree Institute, University of Technology Sydney,

PO Box 123, Broadway,

Sydney, NSW,

Australia, 2007

Email: steven.djordjevic@uts.edu.au

ABSTRACT

ST58 phylogroup B1 *E. coli* have been isolated from a wide variety of mammalian and avian hosts but are not noted for their ability to cause serious disease in humans or animals. Here we determined the genome sequences of two multiple drug resistant ST58 *Escherichia coli* strains from urine and blood of one patient using a combination of Illumina and Single Molecule, Real-Time (SMRT) sequencing. Both ST58 strains were clonal and were characterised as serotype O8:H25 phylogroup B1 and carried a complex resistance locus/loci (CRL) that featured an atypical class 1 integron with a *dfrA5* (trimethoprim resistance) gene cassette followed by only 24 bp of the 3´-CS. CRL that carry this particular integron have been described previously in *E. coli* from cattle, pigs and humans in Australia. The integron abuts a copy of Tn*6029,* an IS*26*-flanked composite transposon encoding *bla*TEM, *sul2, strAB* genes that confer resistance to ampicillin, sulphathiazole and streptomycin respectively. The CRL resides within a novel Tn*2610*-like hybrid Tn*1721*/Tn*21* transposon on an IncF, colV plasmid (pSDJ2009-52F) of 138,553 bp that encodes virulence associated genes (VAGs) implicated in life threatening ExPEC infections. Notably, pSDJ2009-52F shares high sequence identity with pSF-088-1, a plasmid reported in an *E. coli* ST95 from a patient with blood sepsis from a hospital in San Francisco. Our data suggest that extraintestinal infections caused by *E. coli* that carry colV-like plasmids, irrespective of their phylogroup or MLST, may pose a potential threat to human health, particularly to the elderly and the immunocompromised.

Keywords: *E. coli* ST58, ExPEC; virulence plasmid; complex resistance region; Tn*6029,* Tn*2610*

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a phylogenetically diverse group of *E. coli* that have acquired the ability to colonise anatomical sites distinct from the gastrointestinal tract including the urinary tract, brain and spinal cord, soft tissue sites and bone. ExPEC are classified into three subtypes that include uropathogenic *E. coli* (UPEC), neonatal meningitis associated *E. coli* (NMEC) and avian pathogenic *E. coli* (APEC) [1]. ExPEC also cause nosocomial bloodstream infections in hospitals and nursing homes and are often responsible for respiratory infections and bacteraemia in long-term hospitalized patients. ExPEC have been identified in humans, food animals, retail meat products, companion animals and effluent and there is a significant body of circumstantial evidence to suggest that a subset of ExPEC are zoonotic pathogens [2-4].

Our understanding of the evolution and spread of multiple antibiotic resistance in ExPEC has been influenced by efforts to gauge the prevalence of *E. coli* expressing extended spectrum β-lactamases (ESBL), Amp C β-lactamases and plasmid-mediated quinolone and fluoroquinolone resistance. Knowledge of the extent of the carriage of antibiotic resistance genes is likely to be underestimated because many studies restrict phenotypic screening to antibiotics that are of clinical relevance for infection control. As a consequence, resistance to older first generation antibiotics, many of which are still used in veterinary medicine and food production more broadly; particularly ampicillin, sulfonamides, tetracycline and trimethoprim, is ignored.

IS*26* plays a critical role in the capture and dissemination of broad classes of antimicrobial resistance genes including genes encoding resistance to all last line antibiotics. IS*26* is widespread in commensal and pathogenic *E. coli* from food animals in Australia [5, 6] and it often localizes to sites that are within close genetic proximity to class 1 integrons and facilitates formation of complex resistance loci (CRL) on transposons and plasmids [5, 7-13]. IS*26* can promote the generation of hybrid plasmids carrying antibiotic resistance and virulence genes [14], as well as influence plasmid fitness by deleting regions of DNA that encode genes whose expression incurs a cost to the host [15]. Furthermore, CRL containing existing copies of

IS*26* are targeted by mobile genetic cargo flanked by IS*26* enabling host bacteria to adapt rapidly to an antimicrobial selection pressure. Clearly it is important to understand how CRL encoding resistance to first generation antibiotics, and the mobile elements that carry them, have evolved and to determine how widespread they are in commensal and pathogenic *E. coli* and other Gram negative pathogens that are exposed to antimicrobial selection pressures in human healthcare, food animal production settings and the environment.

Here we characterized two UPEC isolates from an elderly patient collected four days apart. Strain 2009-49 was from midstream urine and strain 2009-52 was from blood. The aim of this study was to use whole gene sequencing (WGS) to determine the genetic relation they shared to one another and to isolates belonging to the same MLST, their repertoire of antibiotic resistance and virulence associated genes, and the plasmids they carry.

2. Materials and Methods

2.1 Bacterial strains

Both ST58 strains from this study were sourced from Sydney Adventist Hospital in 2009. Strain 2009-49 was cultured from the mid-stream urine of an elderly patient presenting with a urinary tract infection and strain 2009-52 was cultured from a blood sample obtained from the same patient four days later. The pathology report indicated that the patient had elevated leukocytes and red blood cells and a high bacterial colony count. The urinary tract isolate 2009-49 displayed resistance to ampicillin, trimethoprim and nitrofurantoin while the blood isolate was recorded as being resistant to ampicillin.

2.2 Calibrated Dichotomous Sensitivity (CDS) Testing

Antibiotic susceptibility testing was performed using the established CDS protocol. Strains 2009-49 and 2009-52 and the three reference strains *E. coli* ACM 5185 and 5186 and *Pseudomonas aeruginosa* ACM 5189 137 were grown overnight on LB agar plates at 37°C. Antibiotic discs were applied to Sensitest (Oxoid®) plates at a maximum of 6 per plate using a 6-Cartridge Disc Dispenser (Oxoid®). Strains were tested against 20 antibiotics. These include ampicillin (25 μg), apramycin (15 μg), augmentin

(amoxicillin/clavulanate; 6 µg), azithromycin (15 µg), cefotaxime (5 µg), cefoxitin (30 µg), cephalexin (100 µg), chloramphenicol (30 µg), ciprofloxacin (2.5 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (50 µg), nalidixic acid (30 µg), neomycin (30 µg), nitrofurantoin (200 µg), streptomycin (25 µg), sulfafurazole (300 µg), tetracycline (10 µg), timentin (ticarcillin/clavulanate; 85 µg) and trimethoprim (5 µg).

2.3 Whole Genome Sequencing and Analyses

Whole genome sequencing was performed using an Illumina® MiSeq platform at the ithree institute, University of Technology Sydney following published protocols and raw reads were assembled using the A5 *de novo* assembly pipeline [16]. Sequence typing was performed electronically using the *E. coli* Achtman MLST scheme available at https://pubmlst.org/. Plasmid classification was determined by submission of whole genomes to PlasmidFinder 1.2 (https://cge.cbs.dtu.dk/services/PlasmidFinder/).

SMRT sequencing was performed on strain 2009-52 using a Pacific Biosystems RSII instrument and HGAP assembly at the Ramaciotti Centre at the University of New South Wales, Sydney, Australia. The sequence was polished using Pilon (https://github.com/broadinstitute/pilon). A phylogenetic tree was constructed with 326 complete genome sequences of *E. coli* obtained from Enterobase. PhyloSift v1.0 (http://phylosift.wordpress.com/) was used to undertake the phylogenetic analysis and the output was manipulated using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Phylosift analysis was used to determine the genome sequence most closely related to ST58 strains 2009-49 and 2009-52.

2.4 Single Nucleotide Polymorphisms (SNP)

Parsnp from the Harvest Tools suite (https://harvest.readthedocs.io/en/latest/content/harvest-tools.html) was used to identify SNPs in the core genomes of 2009-49 (Miseq) and 2009-52 (PacBio), by aligning the genomes with the most closely related complete reference genome, SCK30-22, available in Enterobase. x flag was implemented to filter out recombination events, which resulted in alignment of 82.2% of the three genomes. Gingr [17] was used to visualise the SNP locations in the .ggr file and the matching co–ordinates confirmed from the exported .vcf file. The genes affected by SNPs identified in the process was thereafter

confirmed by BLASTn analysis and subjected to SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html) analysis to assess functional alterations in protein function.

Nucleotide sequence accession numbers

Strains 2009-49 and 2009-52 and plasmid pSDJ2009-52F have been submitted to GenBank under accessions NXEP00000000, NXEO00000000 and MH195200 respectively.

3. Results and Discussion

Uropathogenic *E. coli* strain 2009-49 (urine isolate) and 2009-52 182 (blood isolate) were sourced from the same patient within a narrow time frame (4 days). Available metadata indicated both strains were resistant to ampicillin, trimethoprim and nitrofurantoin. CDS assays revealed both strains showed reduced susceptibility to ampicillin, trimethoprim, sulfafurazole, tetracycline and streptomycin. eMLST analysis identified both strains as ST58, a sequence type that resides within the ST155 clonal complex. Both strains were Clermont phylotype B1, were serotyped *in silico* as O8:H25, and carried plasmid incompatibility markers F and FIB. Strain 2009-52 also carried an I2 replicon.

3.1 Whole Genome Sequencing and Comparative Genomic Analyses

Assembly statistics for the two genomes are presented in Table S1. A proportional phylogenetic cladogram generated using Phylosift, comprising genome sequences of isolates 2009-49 and 2009-52 and 84 ST58 *E. coli* genomes of human and animal origin from Enterobase is shown in Figure 1. A more comprehensive phylogenetic tree of ST58 genomes is shown in Figure S2. 2009-49 and 2009-52 clustered together with a node confidence value of 1. The closest relative to the Sydney ST58 strains was strain SCK30-22, an ST58, serotype O8:H25 *E. coli*, sourced from an undisclosed human infection from the Netherlands. Strain SK30-22 was used to extract the core genome of our two ST58 strains and identify SNPs between them. Only four SNPs, all but one (*fimG*; S167R) of which were silent, was identified using Parsnp and Gingr from the Harvest Tools suite. Three SNPs that 2009-52 housed in comparison to 2009-49 and the reference, were located in DNA ligase B, intergenic sequence and the *fimG* gene respectively. One SNP was identified

within a predicted transposase in strain 2009-49. The low number of SNPs is consistent with the two Sydney ST58 strains being clonal. SMRT sequencing showed that strain 2009-52 had a chromosome comprising 5,012,036 nucleotides and two plasmids: IncI2 plasmid pSDJ2009-52I2 (215,832 bp) and IncF plasmid pSDJ2009-52F (138,553 bp).

A whole genome BLASTn analysis using strain 2009-52 genome as the query and 2009-49 (Figure S3) identified a 215,832 nt plasmid (pSDJ2009-52I2) scaffold (Figure S4) in strain 2009-52 but not 2009-49. pSDJ2009-52I2 carries a putative Type IV secretion system (T4SS) (*virD4*, *virB1*, *virB2*, *virB4-B6* and *vir8-11*), phage related genes, pilin genes (*pilN*, *pilO*, *pilP*, *pilQ*, *pilR*, *pilS*, *pilT*, *pilU* and *pilV*), and 36 hypothetical genes. Coding sequences in pSDJ2009-5212 are shown in Table S5.

3.2 Virulence associated genes (VAGs) in ST58 strains

Strains 2009-49 and 2009-52 carry fimbrial adhesins including *bmaE*, *fimH*, *gafD*, *csgG* and *yfcV*, multiple iron acquisition operons, several copper resistance genes, toxins including *hlyE*, *hlyF* and *hek*, and various membrane-associated pumps and proteases (Table 1).

3.3 Characterisation of the complex resistance locus (CRL) in the Sydney ST58 strains

Both 2009-49 and 2009-52 possess an atypical class 1 integron carrying a *dfrA5* gene cassette (trimethoprim resistance) missing all but 24 bp of the 3´-CS. Genetic signatures such as these can be useful to track the plasmids and transposons [5, 10]. A PCR with a forward primer in *intI1* and a reverse primer in IS*26*, developed previously to characterise atypical class 1 integrons [5] generates an amplicon of 848 bp. An identical 848 bp signature is observed in derivate Tn*21*-associated CRL of MDR O26 enterohaemorrhagic *E. coli* (EHEC) from a human and serologically diverse atypical enteropathogenic *E. coli* (EPEC) from Australian cattle [5, 12, 13] and in commensal *E. coli* from swine [6]. The presence of the same signature in UPEC suggests that the Tn*21* transposon, and the plasmids that carry it, are disseminated widely in animal production systems in Australia. The molecular signature is created by an IS*26*-mediated deletion caused by insertion of IS*26* [5] or IS*26*-flanked transposons such as Tn*6029*, into the backbone of mercury resistance transposons belonging to the Tn*3* family [6, 12, 13]. Tn*6029* encodes the *bla*TEM-1 gene (ampicillin resistance), *strAB* (streptomycin

resistance), and *sul2* (sulfathiazole resistance). In EHEC and EPEC that carry the same molecular signature, Tn*6029*, or related transposon Tn*6026*, abuts the atypical class 1 integron precisely 24 bp into the 3´-CS. In strains 2009-49 and 2009-52 the mercury resistance module of Tn*21* flanks one arm of the resistance region CRL while the other arm comprises a hybrid version of Tn*1721*/Tn*21*, that carries the tetracycline resistance gene *tetA*(A), followed by the transposition module of Tn*21* (Figure 2B). The crossover region resides 373 nucleotides upstream of the class 1 integron IR*i* which notably, has been reported previously in a clinical UPEC strain from Sydney (GenBank accession HM999791.1). The CRL is sufficient to encode resistance to ampicillin, streptomycin, sulfonamides, tetracycline, and trimethoprim, a pattern of resistance not uncommon in Australian food animal production systems [5, 18, 19]. Nitrofurantoin resistance may be attributed to mutations or deletions in the chromosomal *nfsA* and *nfsB* genes. In both strains, *nfsA* was missing the terminal 302 nt and *nfsB* contained 10 SNPs compared to that of the nitrofurantoin sensitive (wild type +) strain K-12 and are sufficient to engender an *nfsAB*$^{+}$ genotype in strains 2009-49 and 2009-52.

*3.4 pSDJ2009-52F carries the Tn*1721*/Tn*21 *hybrid transposon*

The hybrid Tn*1721*/Tn*21* transposon is located in the *repFIIa* gene in pSDJ2009-52F and is flanked by 5 bp direct repeats indicating it, or a progenitor of it, transposed to this site. pSDJ2009-52F carries ExPEC VAGs that have a role in the acquisition of iron including *iutA*, *iucDCBA*, *shiF*, *sitDCBA*, *hlyF*, *etsABC*, *iroBCDE*, *iroN*, as well as *ompT*, *iss* and *cva*C genes (Table 1). Both Sydney ST58 strains carry identical copies of pSDJ2009-52F. BLASTn analysis shows that the sequence flanking either side of hybrid transposon shares significant nucleotide sequence identity with pSF-088-1 (accession number CP012636.1), a plasmid found in an ST95 strain of *E. coli* recovered from a patient with a bloodstream infection at San Francisco General Hospital (Figure 2A). ST95 is a sequence type linked with ExPEC disease in poultry and humans. pSF-088-1 but not pSDJ2009-52F contains the full *tra* region and lacks the *tet* genes of Tn*1721*. Notably, pSF-088-1 carries the same atypical class 1 integron found in the uropathogenic Sydney ST58 strains.

4. Conclusion

Here we describe the isolation and sequence analysis of two *E. coli* ST58 phylotype B1 strains from the urine and blood of the same patient. Both ST58 strains carry pSDJ2009-52F, an IncF plasmid that harbours a

CRL encoding multiple antibiotic resistance genes, IS*26*, and a variety of VAGs that are typical of colV plasmids. ColV plasmids have been implicated in ExPEC infections in human and animals often causing urinary, neurological and systemic disease. pSDJ2009-52F encodes resistance to multiple classes of antibiotics, the genes of which are housed within a Tn*2610*-like hybrid Tn*1721*/Tn*21* transposon.

E. coli ST58 has been isolated from mammalian and avian hosts including European rooks, humans, cats, camels and cattle. In Enterobase (https://enterobase.warwick.ac.uk/species/index/ecoli), ST58 strains are described from a range of sources including humans, pigs, cows, horses, retail meat, animal feed, dogs, turkeys and various other birds, a prairie dog, goats, sheep, spinach, water and soil and various geographic locations including Australia, Ireland, France, Denmark, Tanzania, Ethiopia, Japan, Poland, Thailand, Sweden, Belgium, Canada, Spain, the Netherlands, USA, Brazil, Germany, the United Kingdom, China, and Bangladesh (accessed 06/06/2018). To our knowledge this is the first description of a multiple antibiotic resistant ST58 strain associated with both UTI and blood sepsis. Our studies provide an example of how acquisition of a single plasmid can impart an arsenal of virulence and antibiotic resistance genes to a commensal *E. coli* with a wide host range and suggests that the acquisition of the IncF plasmid pSD2009-52F is significant in the evolution of our Sydney ST58 strains and potentially, their ability to cause UTI and urosepsis.

Acknowledgments

This work is supported by Ausgem a collaborative partnership between the New South Wales Department of Primary Industries' Elizabeth Macarthur Agricultural Institute (EMAI) and the ithree institute at the University of Technology Sydney (UTS). The authors thank Nola Hitchick and Natalie Miller for providing the *Escherichia coli* ST58 strains from the SAN Hospital. We also thank Ethan Wyrsch for assistance with bioinformatics analyses and Jaqueline Melvold for assistance with analysis of hypothetical proteins.

Declarations

Funding: This work is supported by AUSGEM. JM is the recipient of an Australian Government Research Training Program Scholarship.

Competing Interests: None to declare.

Ethical Approval: Not required

References

1. Pitout JD. Extraintestinal Pathogenic Escherichia coli: A Combination of Virulence with Antibiotic Resistance. *Front Microbiol* 2012; **3**: 9.
2. Manges AR. Escherichia coli and urinary tract infections: the role of poultry-meat. *Clin Microbiol Infect* 2016; **22**: 122-9.
3. Nordstrom L, Liu CM, Price LB. Foodborne urinary tract infections: a new paradigm for antimicrobial-resistant foodborne illness. *Front Microbiol* 2013; **4**: 29.
4. Vincent C, Boerlin P, Daignault D et al. Food reservoir for Escherichia coli causing urinary tract infections. *Emerg Infect Dis* 2010; **16**: 88-95.
5. Dawes FE, Kuzevski A, Bettelheim KA et al. Distribution of class 1 integrons with IS26-mediated deletions in their 3′-conserved segments in Escherichia coli of human and animal origin. *PloS one* 2010; **5**: e12754.
6. Reid CJ, Wyrsch ER, Roy Chowdhury P et al. Porcine commensal Escherichia coli: a reservoir for class 1 integrons associated with IS26. *Microbial genomics* 2017; **3**.
7. Cain AK, Liu X, Djordjevic SP et al. Transposons related to Tn1696 in IncHI2 plasmids in multiply antibiotic resistant Salmonella enterica serovar Typhimurium from Australian animals. *Microb Drug Resist* 2010; **16**: 197-202.
8. Levings RS, Lightfoot D, Partridge SR et al. The genomic island SGI1, containing the multiple antibiotic resistance region of Salmonella enterica serovar Typhimurium DT104 or variants of it, is widely distributed in other S. enterica serovars. *J Bacteriol* 2005; **187**: 4401-9.
9. Reid C, Roy Chowdhury P, Djordjevic SP. *Tn6026 and Tn6029 are found in complex resistance regions mobilized by diverse plasmids and chromosomal islands in multiple antibiotic resistant Enterobacteriaceae*, 2015.
10. Roy Chowdhury P, Charles IG, Djordjevic SP. A role for Tn6029 in the evolution of the complex antibiotic resistance gene loci in genomic island 3 in enteroaggregative hemorrhagic Escherichia coli O104:H4. *PLoS One* 2015; **10**: e0115781.
11. Saenz Y, Vinue L, Ruiz E et al. Class 1 integrons lacking qacEDelta1 and sul1 genes in Escherichia coli isolates of food, animal and human origins. *Vet Microbiol* 2010; **144**: 493-7.
12. Venturini C, Beatson SA, Djordjevic SP et al. Multiple Antibiotic Resistance Gene Recruitment onto the Enterohemorrhagic Escherichia coli Virulence Plasmid. *FASEB J* 2010; **24**: 1160-6.
13. Venturini C, Hassan KA, Roy Chowdhury P et al. Sequences of two related multiple antibiotic resistance virulence plasmids sharing a unique IS26-related molecular signature isolated from different Escherichia coli pathotypes from different hosts. *PLoS One* 2013; **8**: e78862.
14. Mangat CS, Bekal S, Irwin RJ et al. A Novel Hybrid Plasmid Carrying Multiple Antimicrobial Resistance and Virulence Genes in Salmonella enterica Serovar Dublin. *Antimicrobial Agents and Chemotherapy* 2017; **61**: e02601-16.
15. Porse A, Schønning K, Munck C et al. Survival and evolution of a large multidrug resistance plasmid in new clinical bacterial hosts. *Molecular biology and evolution* 2016; **33**: 2860-73.
16. Darling AE, McKinnon J, Worden P et al. A draft genome of Escherichia coli sequence type 127 strain 2009-46. *Gut pathogens* 2014; **6**: 32.
17. Treangen TJ, Ondov BD, Koren S et al. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biology* 2014; **15**: 524.
18. Abraham S, Jordan D, Wong HS et al. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant Escherichia coli in Australian food-producing animals. *Journal of global antimicrobial resistance* 2015; **3**: 273-7.
19. Bettelheim KA, Hornitzky MA, Djordjevic SP et al. Antibiotic resistance among verocytotoxigenic Escherichia coli (VTEC) and non-VTEC isolated from domestic animals and humans. *J Med Microbiol* 2003; **52**: 155-62.

Figure and Table legends:

Figure 1. Proportional cladogram of all ST58 genomes from Enterobase with complete metadata according to our selection criteria. A black circle indicates a node value of 1.

Figure 2. A) Sequence of pSDJ2009-52F as compared to pSF-088-1 via BRIG. Green/purple histogram is GC skew, black histogram is GC content and black bar is BLAST match with pSF-088-1. Outer ring is a schematic of pSDJ2009-52F created in SnapGene. Virulence-associated genes and the CRL are colour-coded. B) Complex resistance loci found in ST58 strains 2009-49 and 2009-52. Genes are colour coded to coincide with those in Figure 2A.

Table 1. Putative virulence factors in ST58 strains 2009-49 and 2009-52

Table 1.

Gene Function		Gene Name	% Identity	Location
Metal Resistance, Transport and Synthesis	Yersiniabactin receptor	*chuA*	98	chromosome
	Control of copper homeostasis	*copA*	98	chromosome
	multicopper oxidase	*cueO*	97	chromosome
	Transcriptional regulator	*cueR*	100	chromosome
	Copper and silver efflux membrane component	*cusA*	99	chromosome
	Copper and silver efflux membrane fusion protein	*cusB*	99	chromosome
	Copper and silver efflux outer membrane protein	*cusC*	98	chromosome
	Periplasmic copper binding protein	*cusF*	98	chromosome
	Response regulator	*cusR*	97	chromosome
	Histidine kinase	*cusS*	97	chromosome
	Putative type 1 secretion membrane-fusion protein	*etsA*	99	pSDJ2009-52F
	Putative type 1 secretion ATP binding protein	*etsB*	99	pSDJ2009-52F
	Putative type 1 secretion outer membrane protein	*etsC*	100	pSDJ2009-52F
	Pesticin/Yersiniabactin receptor	*fyuA*	99	chromosome
	Iron-related glycosyltransferase	*iroB*	100	pSDJ2009-52F
	Putative ABD transporter	*iroC*	99	pSDJ2009-52F
	Ferric enterochelin esterase	*iroD*	99	pSDJ2009-52F
	Siderophore esterase	*iroE*	99	pSDJ2009-52F
	Iron outer membrane receptor	*iroN*	100	pSDJ2009-52F
	Yersiniabactin biosynthesis	*irp1*	99	chromosome
	Yersiniabactin biosynthesis	*irp2*	99	chromosome
	Aerobactin biosynthesis	*iucA*	100	pSDJ2009-52F
	Aerobactin biosynthesis	*iucB*	100	pSDJ2009-52F
	Aerobactin biosynthesis	*iucC*	100	pSDJ2009-52F
	Aerobactin biosynthesis	*iucD*	99	pSDJ2009-52F
	Ferric aerobactin receptor	*iutA*	100	pSDJ2009-52F
	Iron/manganese transport system periplasmic binding protein	*sitA*	99	pSDJ2009-52F
	Iron/manganese transport system inner membrane protein	*sitB*	100	pSDJ2009-52F
	Iron/manganese transport system inner membrane protein	*sitC*	99	pSDJ2009-52F
	Iron/manganese transport system inner membrane protein	*sitD*	100	pSDJ2009-52F
Colicins and Colicin Immunity	Colicin V secretion protein	*cvaA*	100	pSDJ2009-52F
	Colicin V secretion protein	*cvaB*	99	pSDJ2009-52F
	Colicin V precursor	*cvaC*	100	pSDJ2009-52F
	Colicin V immunity protein	*cvi*	100	pSDJ2009-52F
Adhesion	Phase-variable biofilm formation autotransporter	*agn43*	98	chromosome
	M-agglutinin subunit	*bmaE*	100	chromosome
	Curli secretion channel	*csgG*	98	chromosome
	Tyrosine recombinase	*fimB*	99	chromosome
	Minor fimbrial subunit	*fimH*	99	chromosome
	G-fimbriae	*gafD*	99	chromosome
	putative fimbrial-like adhesin protein	*yfcV*	98	chromosome
Blood-associated	Auto-aggregating adhesin and invasion	*hek*	98	chromosome
	Haemolysin/cytolysin	*hlyE*	99	chromosome
	Avian haemolysin and regulator of outer membrane vesicle biogenesis	*hlyF*	100	pSDJ2009-52F

	Increased serum survival and resistance to complement	*iss*	100	pSDJ2009-52F
Other	Outer membrane protease, regulates the biogenesis of outer membrane vesicles	*ompT*	100	pSDJ2009-52F
	putative membrane transport protein	*shiF*	99	pSDJ2009-52F
	Conjugal transfer surface exclusion protein	*traT*	99	pSDJ2009-52F

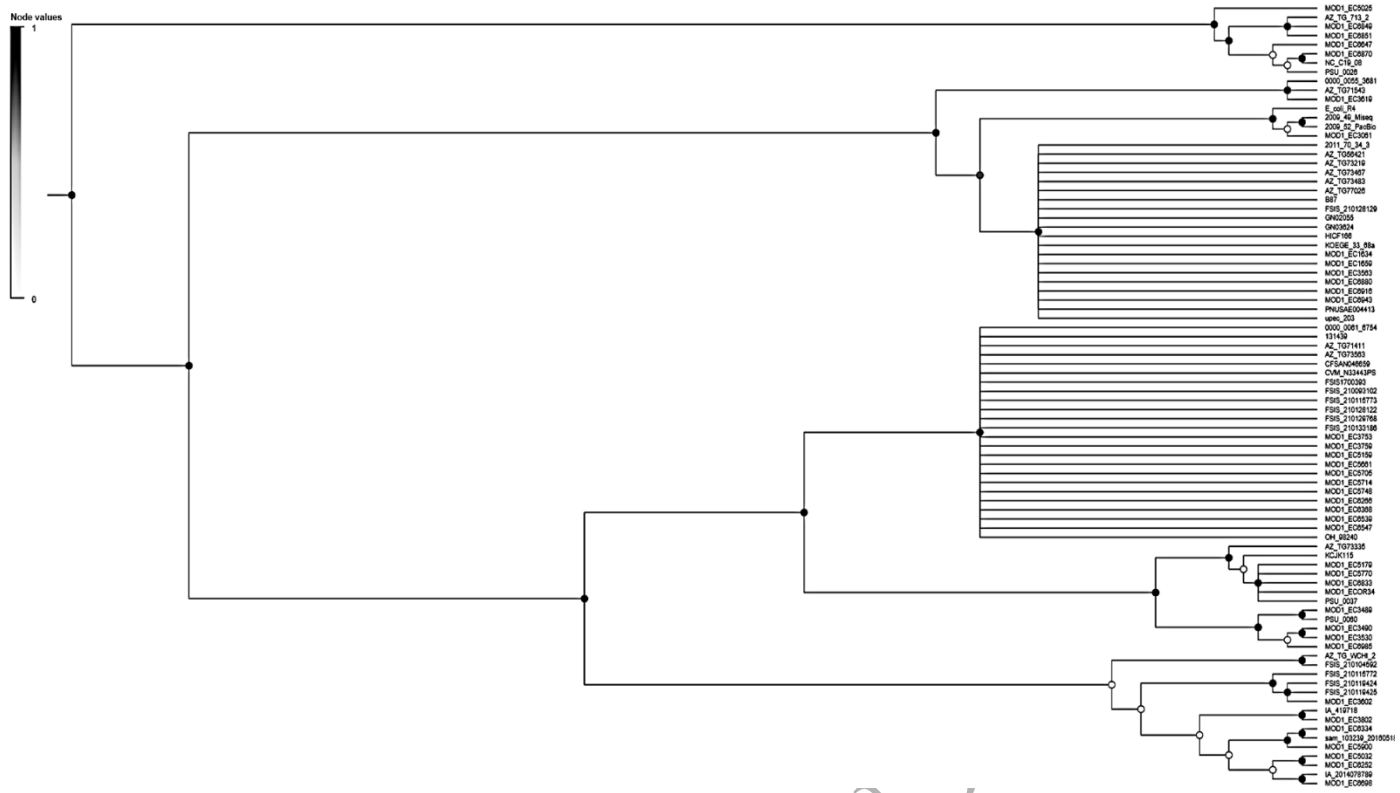

Figure 1.

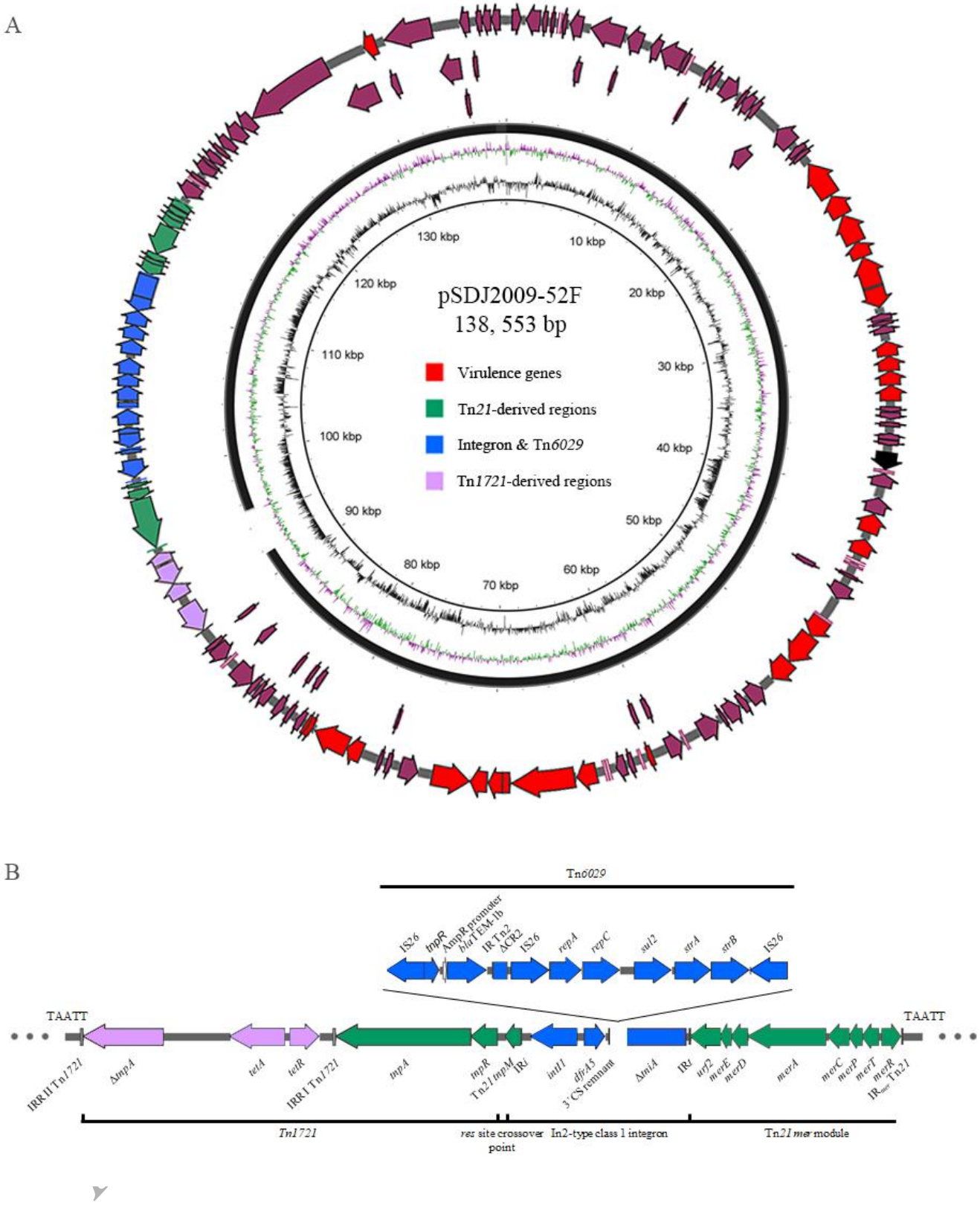
A
pSDJ2009-52F
138, 553 bp
Virulence genes
Tn21-derived regions
Integron & Tn6029
Tn1721-derived regions
10 kbp
20 kbp
30 kbp
40 kbp
50 kbp
60 kbp
70 kbp
80 kbp
90 kbp
100 kbp
110 kbp
120 kbp
130 kbp
B
Tn6029
IS26
tnpR
AmpR promoter
blaTEM-1b
IR Tn2
ΔCR2
IS26
repA
repC
sul2
strA
strB
IS26
TAATT
TAATT
IRR II Tn1721
ΔtnpA
tetA
tetR
IRR I Tn1721
tnpA
tnpR Tn21tnpM
IRi
intI1
dfrA5
3′CS remnant
ΔtniA
IRi
urf2
merE
merD
merA
merC
merP
merT
merR
IRmer Tn21
Tn1721
res site crossover point
In2-type class 1 integron
Tn21 mer module

Figure 2.