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Genome Note

Genome sequence of a clinical *Salmonella* Enteritidis sequence type 11 strain from South Africa

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ABSTRACT

Objectives: The underlying resistance mechanism and phylogenetic relationship of a colistin-resistant *Salmonella enterica* serovar Enteritidis strain EC20120916 that resulted in fatal meningitis in an immunocompromised patient was investigated by whole-genome sequencing (WGS) analysis.
Methods: WGS of strain EC20120916 was performed on an Illumina MiSeq platform and annotation of the sequence was performed using the Prokaryotic Genome Annotation Pipeline (PGAP). Antimicrobial resistance genes, plasmid replicons and pathogenicity islands were identified. A phylogenetic tree was constructed using Parsnp and was edited with FigTree.
Results: The genome size of strain EC20120916 is 4 699 318 bp with a GC content of 55.2% and 4471 protein-coding genes. The *aac(6′)-Iaa* gene, encoding resistance to aminoglycosides, was identified although this was not expressed phenotypically in the isolate. No colistin resistance-conferring mutations or plasmid-mediated mechanisms were identified to explain the colistin resistance. The strain was phylogenetically related to three international strains, although it was not close enough to suggest importation from outside of South Africa.
Conclusion: This is the first report of a colistin-resistant *Salmonella* Enteritidis isolate causing meningitis in an immunocompromised patient in South Africa. The absence of colistin resistance-conferring mutations or plasmid-mediated resistance mechanisms suggest that a novel mechanism is responsible for the colistin resistance in this isolate. The isolate was acquired locally.

Salmonellae belong to the Enterobacteriaceae family of Gram-negative bacilli [1]. The species is comprised of *Salmonella enterica* and *Salmonella bongori*. The species *S. enterica* consists of typhoidal serovars, namely *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi as well as various other non-typhoidal *Salmonella* (NTS) serovars [2]. *Salmonella enterica* serovar Enteritidis is among the most frequently isolated NTS causing invasive disease in Africa [3]. NTS are known commensals of the gastrointestinal tract of many animals, with *Salmonella* Enteritidis being especially associated with poultry flocks [1]. Transmission and subsequent infection most commonly arises through ingestion of contaminated food or water. The infectious dose ranges from 10^3–10^6 organisms [4].

A *Salmonella* Enteritidis sequence type 11 (ST11) strain (EC20120916) was isolated from the cerebrospinal fluid of a 34-year-old immunosuppressed male patient diagnosed with meningitis in a hospital in Pretoria, South Africa, in 2018. Antimicrobial susceptibility testing using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) and the broth microdilution method showed the strain to be resistant to colistin, a last-resort antibiotic agent used against multidrug-resistant Gram-negative bacteria.

Strain EC20120916 was grown aerobically overnight at 35 °C on 5% sheep blood agar, chocolate agar and MacConkey agar. The isolate grew as a flat, dry, non-lactose fermenter, which agglutinated positive for Group D with the Wellcolex™ Color *Salmonella* Agglutination Test Kit (Remel, London, UK). The VITEK®2 automated system confirmed the organism to be *Salmonella* group, and subsequent serotyping and classification according to the Kauffman–White scheme revealed the organism to be *Salmonella* Enteritidis.

Genomic DNA from the isolate was fragmented using an enzyme-based approach. The resulting fragments were purified (size selected),

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end-repaired and an Illumina-specific adapter sequence was ligated to all fragments. Following quantification, the samples were individually indexed and a second size selection step was performed using AMPure XP beads. The libraries were quality controlled on a DNA chip (Agilent 2100 Bioanalyzer) and were then sequenced on an Illumina MiSeq platform (Illumina Inc.).

The genomic features of the isolate were as follows: genome size 4 699 318 bp; 55.2% GC content; 92 contigs; and contig N_{50} and L_{50} values of 117 754 bp and 12 bp, respectively. Annotation with the Prokaryotic Genome Annotation Pipeline (PGAP) [5] found 4471 protein-coding genes, 69 tRNAs, 3 rRNAs (one 16S and two 23S), 14 non-coding RNAs and two clustered regularly interspaced short palindromic repeat (CRISPR) arrays, indicating that the isolate was exposed to bacteriophages. The isolate contains at least three plasmid replicons (Col440I, IncFIB and IncFII), suggesting the presence of at least one plasmid, as determined using PlasmidFinder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) and pMLST 2.0 (https://cge.cbs.dtu.dk/services/pMLST/). Eight pathogenicity islands were also identified using SPIFinder 1.0 (https://cge.cbs.dtu.dk/services/SPIFinder/).

Although strain EC20120916 harbours the *aac(6′)-Iaa* gene, encoding resistance to aminoglycosides, it was phenotypically susceptible to aminoglycosides, with minimum inhibitory concentrations (MICs) for gentamicin and amikacin of ≤1 μg/mL and ≤2 μg/mL, respectively. Uniquely, strain EC20120916 displayed phenotypic resistance to colistin, with MICs of 8 μg/mL and 4 μg/

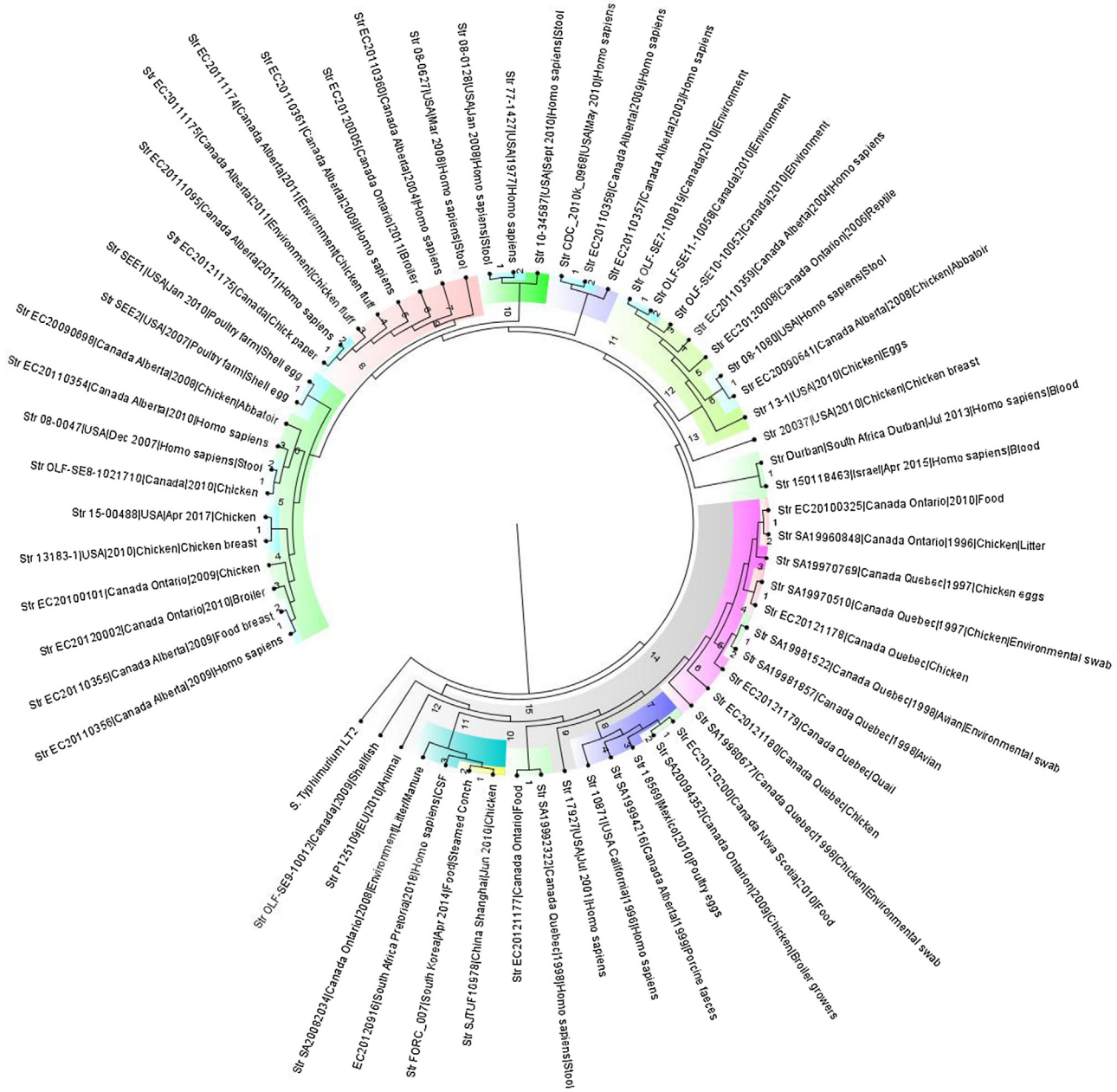

Fig. 1. Phylogenetic tree showing the clonal relationship between *Salmonella enterica* serovar Enteritidis EC20120916 and other *Salmonella* Enteritidis strains of international origin. *Salmonella enterica* serovar Typhimurium LT2 is the reference or outgroup isolate and the branch labels refer to the bootstrap values. Isolates belonging to the same clades and clones are highlighted with the same colours to show their close association. Strain EC20120916 had no closely related clone but is of the same clade as SA20082034, FORC_007 and SJTUF10978 from Canada (Ontario), South Korea and China (Shanghai), respectively. The close relationship between certain international strains shows the evolution and dissemination of *Salmonella* Enteritidis worldwide. On particular, isolates from North America, Canada and the USA are of such a close relationship that it is possible they originated from the same ancestor at some point in time.

mL as determined by VITEK®2 and broth microdilution, respectively. However, genome analysis using the NCBI's BLAST suite found no colistin resistance-conferring mutations in *pmrAB*, *pmrHFIJKLMD*, *arnE*, *arnC*, *phoPQ*, *mgrB* and *acrAB* genes or plasmid-mediated *mcr* genes to explain this resistance. We can therefore conclude that the colistin resistance is due to a novel mechanism, yet to be characterised.

The genetic relatedness of the isolate with other strains was represented in a phylogenetic tree drawn with Parsnp (https://github.com/marbl/parsnp) and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree/) (Fig. 1). Strain EC20120916 is closely related to strains SA20082034, FORC_007 and SJTUF10978 from Canada (Ontario), South Korea and China (Shanghai), respectively, although there is little to suggest that it was imported from abroad. The close relationship between these international strains shows the evolution and dissemination of *Salmonella* Enteritidis worldwide. In particular, isolates from North America, Canada and the USA are in most cases of the same clade, suggesting their evolution from a common ancestor at some point in the past. Another interesting observation is the close sequence similarity between *Salmonella* Enteritidis strain Durban from Durban (South Africa) and strain 150118463 from Israel, suggesting that these two isolates are of the same clone and originated from a common ancestor of *Salmonella* Enteritidis.

This whole-genome analysis provides insight into the pathogenesis and resistance of a unique *Salmonella* Enteritidis strain cultured in a clinical isolate in South Africa.

Nucleotide sequence accession no.

The draft genome sequence of *Salmonella* Enteritidis EC20120916 has been submitted to NCBI/GenBank with the accession no. **SHPL00000000** (BioProject **PRJNA521953**).

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Competing interests

None declared.

Ethical approval

Ethical approval for this study was granted by the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria [ethics reference no. 709/2018]. Informed consent was obtained from the patient's next of kin.

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