Genome Sequence of a Clinical *Salmonella* Enteritidis Sequence Type 11 Strain from South Africa

1. Roxanne Rule[a,b*]

   a. Department of Medical Microbiology, Pathology Building, University of Pretoria, Prinshof Campus, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

   b. Tshwane Academic Division, National Health Laboratory Service, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

   roxyrule@gmail.com

2. Mohamed Said[a,b]

   a. Department of Medical Microbiology, Pathology Building, University of Pretoria, Prinshof Campus, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

   b. Tshwane Academic Division, National Health Laboratory Service, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

   msaid@mweb.co.za

3. Nontombi Mbelle[a,b]

   a. Department of Medical Microbiology, Pathology Building, University of Pretoria, Prinshof Campus, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

   b. Tshwane Academic Division, National Health Laboratory Service, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

nontombi.mbelle@up.ac.za

4. John Osei Sekyere[a]

   a. Department of Medical Microbiology, Pathology Building, University of Pretoria, Prinshof Campus, Corner of Steve Biko Road and Dr Savage Road, Pretoria, South Africa, 0084

jod14139@yahoo.com

* **Corresponding author**: Dr Roxanne Rule, University of Pretoria/National Health Laboratory Service, Medical Microbiology, 5 Bophelo Road, Riviera, 0084; telephone +27 82 833 3071; email roxyrule@gmail.com

Abstract

Objectives:

The underlying resistance mechanism and phylogenetic relationship of a colistin-resistant *Salmonella* Enteritidis strain EC20120916 that resulted in fatal meningitis in an immune-compromised patient was investigated by analysing the genome sequence.

Methods:

Whole-genome sequencing was performed on strain EC20120916 with the Illumina MiSeq platform. Annotation of the sequence was performed using the prokaryotic genome annotation pipeline (PGAP). Antibiotic resistance gene, plasmid replicons and pathogenicity islands were identified. A phylogenetic tree was constructed using Parsnp and edited with Figtree.

Results:

The genome size was 4, 699, 318 bp with a GC content of 55.2% and 4471 protein-encoding genes. The *aac(6')-laa* 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 South Africa.

Conclusion:

This is the first report of a colistin-resistant *Salmonella* Enteritidis isolate causing meningitis in an immune-compromised 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.

Keywords:

Salmonella Enteritidis, meningitis, colistin resistance, whole-genome sequencing, South Africa

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 viz., *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi, and various other non-typhoidal *Salmonella enterica* serovars[a] (NTS).[2] *Salmonella enterica* serovar Enteritidis (*S.* 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 *S.* 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[a] (AST) with Vitek 2 (BioMerieux, France) and broth microdilution showed it to be resistant to colistin, a last-resort antibiotic agent used against multidrug-resistant Gram-Negative bacteria.

EC20120916 was grown overnight aerobically 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 Colour *Salmonella* Agglutination (Remel, London, UK) test. The Vitek 2 (bioMérieux, France) 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 *S.* Enteritidis.

The isolate's gDNA was fragmented using an enzyme-based approach. Resulting fragments were purified (size selected), end-repaired and an Illumina-specific adapter sequence was

[a] AST: Antimicrobial susceptibility testing

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 then sequenced on Illumina MiSeq.

The isolate's genomic features were as follows: 4, 699, 318 bp, 55.2% GC content, 92 contigs, and contig N_{50} and L_{50} values of 117754 bp and 12 bp, respectively. Annotation with the prokaryotic genome annotation pipeline[b] (PGAP)[5] found 4471 protein-encoding genes, 69 tRNAs, three rRNAs (one 16S and two 23S), 14 non-coding RNAs and two clustered regularly interspaced short palindromic repeat[c] (CRISPR) arrays, indicating that the isolate was exposed to bacteriophages. The isolate contains at least three plasmid replicons viz., the Col440I, IncFIB and IncFII, suggesting the presence of at least one plasmid; this was 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 had the *aac(6')-Iaa* gene, encoding resistance to aminoglycosides, it was phenotypically susceptible to aminoglycosides: minimum inhibitory concentration[d] (MIC) values for gentamicin and amikacin were respectively ≤ 1 µg/mL and ≤ 2 µg/mL. Uniquely, strain EC20120916 displayed phenotypic resistance to colistin with MIC values of 8 µg/mL and 4 µg/mL as tested by the Vitek 2 system and broth microdilution, respectively. Genome analysis using NCBI's BLAST suite however, found no colistin

[b] PGAP: Prokaryotic genome annotation pipeline

[c] CRISPR: Clustered regularly interspaced short palindromic repeat

[d] MIC: Minimum inhibitory concentration

resistance-conferring mutations in *pmrAB, pmrHFIJKLMD, arnE, arnC, phoPQ, mgrB* and *acrAB* genes nor 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 is represented in a phylogenetic tree drawn with Parsnp (http://github.com/marbl/harvest) and edited with Figtree (http://tree.bio.ed.ac.uk/software/figtree/) (Figure 1). Strain EC20120916 is closely related to strains SA20082034, FORC_2007 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 *S.* Enteritidis worldwide. Particularly, isolates from North America, Canada and the US, are in most cases of the same clade, suggesting their evolution from a common ancestor at a point in the past. Another interesting observation is the close sequence similarity between *S.* Enteritidis Str Durban from Durban (South Africa) and Str 150118463 from Israel, suggesting that these two isolates are of the same clone and originated from a common ancestor of *S.* Enteritidis.

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

Nucleotide sequence accession number:

The draft genome sequence for this isolate has been submitted to NCBI/GenBank with the accession number **SHPL00000000** (PRJNA PRJNA521953).

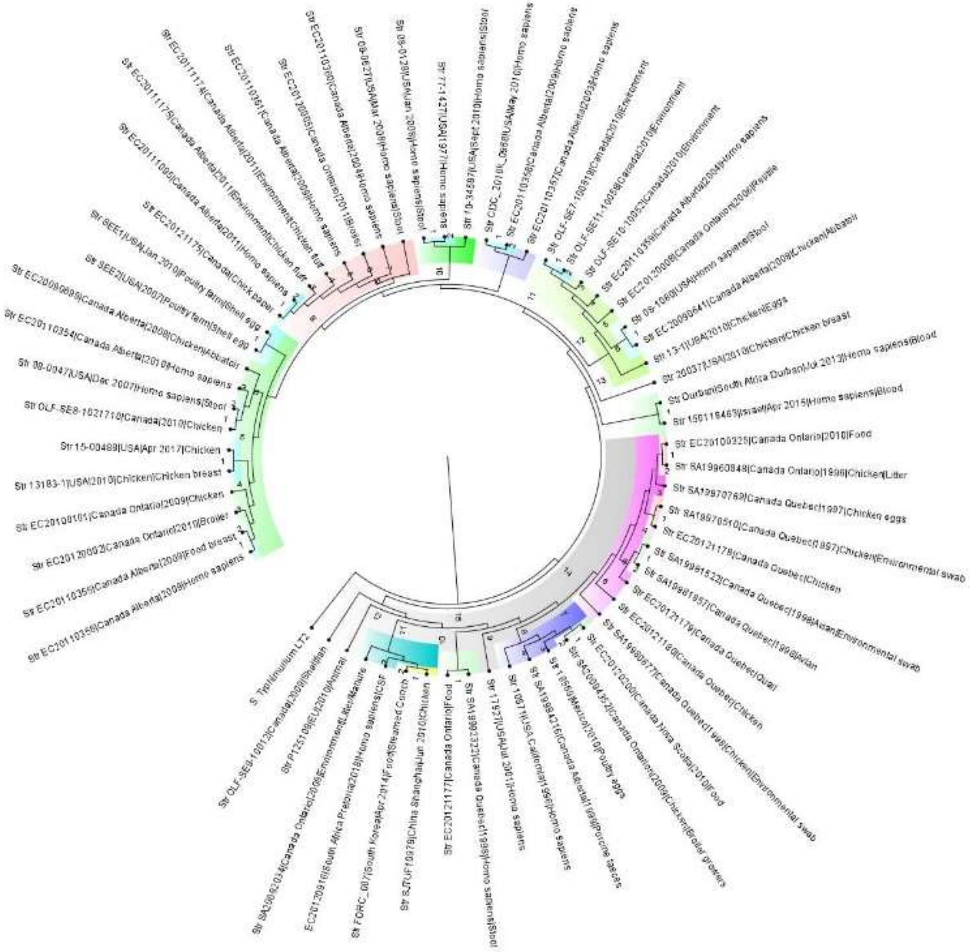

Figure 1. A phylogenetic tree showing the clonal relationship between *S.* Enteritidis EC20120916 and other *S.* Enteritidis strains of international origin. *S.* Typhimurium LT2 is the reference or outgroup isolate and the branch labels refer to the bootstrap values. Isolates belonging to the same clades and clones are highlighted with the same colours to show their close association. The EC20120916 had no closely related clone but is of the same clade as SA20082034, FORC_2007 and SJTUF10978 from Canada (Ontario), South Korea and China (Shanghai) respectively. The close relationship between certain international strains shows the evolution and dissemination of *S.* Enteritidis worldwide. Particularly, isolates from North America, Canada and the US, are of such a close relationship that it is possible they originated from the same ancestor at some point in time.

Funding:

Microscopy, culture and sensitivity testing of EC20120916 was performed as per routine diagnostic laboratory workflow. Whole-genome sequencing of the isolate was funded by research funds available from the Department of Microbiology, University of Pretoria.

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 number: 709/2018. Informed consent was obtained from the patient's next of kin.

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