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Genome Note

Complete genome sequence of a ciprofloxacin-resistant *Salmonella* Kentucky ST198 strain from a chicken carcass in South Korea

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ABSTRACT

Objectives: Human infection by ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky sequence type 198 (ST198) has been reported in the USA, Europe and Korea. In this study, we report the complete genome sequence of the first *Salmonella* Kentucky ST198 strain isolated from a chicken carcass in South Korea.

Methods: The recovered *Salmonella* Kentucky, designated as K13SK002, was isolated from a chicken carcass in 2013. Genomic DNA was sequenced using a combination of 20-kb PacBio SMRTbell library and PacBio RS II. Antimicrobial susceptibility testing was performed and minimum inhibitory concentration (MIC) values were determined. Antimicrobial resistance and virulence genes were investigated in silico using ResFinder and VirulenceFinder tools, respectively, available at the Center for Genomic Epidemiology server.

Results: The genome of K13SK002 consists of contiguous sequences (contigs) with a total length of 4 847 849 bp and a GC content of 52.20%. We detected a total of 4352 protein-coding sequences, 85 tRNA genes and 22 rRNA genes. MICs for ampicillin, ciprofloxacin, gentamicin, streptomycin, sulfisoxazole and tetracycline were 64, 16, 16, 64, >256 and 128 μg/mL, respectively. We found six antimicrobial resistance genes, however no plasmids and genes associated with adherence, toxins and exoenzyme were found. Ciprofloxacin-resistant *Salmonella* Kentucky K13SK002 was found to have mutations in DNA gyrase A (S83F and D87Y).

Conclusion: This is the first report of the complete genome sequence of a *Salmonella* Kentucky ST198 strain isolated from a chicken carcass in South Korea. This genome sequence provides useful information on the genomic features associated with virulence and antimicrobial resistance in *Salmonella* Kentucky ST198.

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While most cases of foodborne salmonellosis are associated with diarrhoea, some lead to hospitalisation and death [1]. Fluoroquinolones and cephalosporins are the first-line drugs used for treating such infections [2]. In South Korea, enrofloxacin, a fluoroquinolone, has been heavily used in poultry production since its approval in the 1990s. Recently, fluoroquinolone-resistant *Salmonella enterica* serovar Kentucky emerged and spread worldwide [3]. Ciprofloxacin-resistant *Salmonella* Kentucky sequence type 198 (ST198) is increasingly frequent in humans, poultry and chicken products in Southeast Asia and Europe, threatening public health and food safety [3]. Human infections caused by this strain have been reported among Koreans who have travelled to Southeast Asia [4]. However, as yet there are no reports of livestock-associated *Salmonella* Kentucky ST198 in South Korea. Here we report the first complete genome sequence of a ciprofloxacin-resistant *Salmonella* Kentucky ST198 strain, designated K13SK002, isolated from chicken in order to understand its genomic features, particularly the presence of antimicrobial resistance genes.

Ciprofloxacin-resistant *Salmonella* Kentucky strain K13SK002 was isolated in 2013 from a chicken carcass from a slaughterhouse chilling room in Incheon Province using the Korean Veterinary Antimicrobial Resistance Monitoring System. Following enrichment on tryptic soy agar at 37 °C (Becton Dickinson, Sparks, MD,

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USA), the isolate was identified as *Salmonella* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDT-TOF/MS) (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility was performed by the microplate dilution method using a commercial system (Sensititre; TREK Diagnostic Systems, West Sussex, UK). Minimum inhibitory concentration (MIC) values for ampicillin, ciprofloxacin, gentamicin, streptomycin, sulfisoxazole and tetracycline were determined using Panel KRNV5 (TREK Diagnostic Systems).

For PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) sequencing, 8 μg of input genomic DNA was used for a 20-kb library preparation. The library insert sizes were optimal; gDNA was sheared with g-TUBE (Covaris Inc., Woburn, MA, USA) and was purified using AMPure® PB magnetic beads (Beckman Coulter Inc., Brea, CA, USA) if the apparent size was >40 kb. Hierarchical Genome Assembly Process (HGAP) 2 package contained in the SMRT v.2.3.0 software was used for de novo assembly and consensus polishing. Next, assembly data were circularised using Circulator 1.4.0. Glimmer3 [5] was used to predict genes, wherein annotation was performed using a homology-based search in the Clusters of Orthologous Groups (COG) database. Antimicrobial resistance and virulence genes were analysed using ResFinder v.4.0 and VirulenceFinder v.2.0 webtools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). We used the default settings for gene identity threshold (95%) and minimum length (60%).

A single contig (N_{50} = 4 847 849 bp) was generated by assembling reads from the PacBio SMRT Analysis 2.3.0 system. The genome of strain K13SK002 was 4 847 849 bp with an average coverage of 204.16× and a GC content of 52.20%. The genome contained 4352 predicted protein-coding sequences, 84 tRNA genes and 22 rRNA genes. The genome annotation is shown in Fig. 1. Regarding COG distribution, S (function unknown, 1233 ORFs), G (carbohydrate transport and metabolism, 384 ORFs), E (amino acid transport and metabolism, 378 ORFs), K (transcription, 333 ORFs), C (energy production and conversion, 295 ORFs) and M (cell wall/membrane/envelope biogenesis, 283 ORFs) were the most abundant categories (>6% of total COG-matched counts). The *Salmonella* isolate, initially identified as *Salmonella* Kentucky, was further confirmed by sequencing. Molecular typing revealed that it belonged to ST198 (allelic profile 76-14-3-77-64-64-67).

MICs for ampicillin, ciprofloxacin, gentamicin, streptomycin, sulfisoxazole and tetracycline were 64, 16, 16, 64, >256 and 128 μg/mL, respectively. Thus, strain K13SK002 was found to be resistant to these antimicrobials. This resistance pattern was consistent with its genetic background, wherein sequence analysis revealed that strain K13SK002 harboured genes encoding aminoglycoside-modifying enzymes [*aac(3)-Id*, *aac(6′)-Iaa*, *aadA7*], β-lactam resistance (bla_{TEM-1B}), sulphonamide resistance (*sul1*) and tetracycline resistance (*tetA*).

In addition, we identified two mutations in the DNA gyrase gene *gyrA* (S83F and D87Y) previously reported to be associated with fluoroquinolone resistance [4]. However, virulence genes encoding adherence-associated proteins, toxins, exoenzymes and extrachromosomal plasmids, which are commonly found in highly resistant micro-organisms, were not detected in K13SK002.

In conclusion, although K13SK002 likely originated from poultry, cross-contamination during meat processing cannot be excluded. Nevertheless, this is the first report of the complete

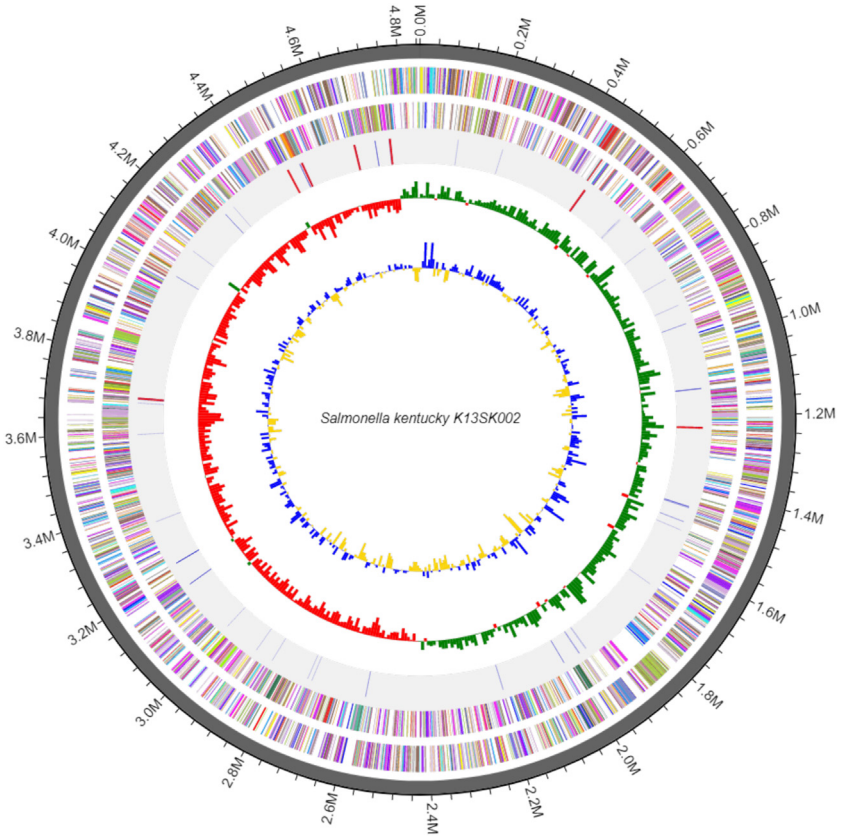

Fig. 1. Genome map of *Salmonella enterica* serovar Kentucky ST198 strain K13SK002. Black circle: genome size; outmost circle: annotated reference gene in the forward coding sequence (CDS); middle circle: annotated reference genes in the reverse CDS; inner circle: rRNAs and tRNAs; red and green lines: GC skew; blue and yellow lines: GC ratio.

genome sequence of a *Salmonella* Kentucky ST198 strain isolated from a chicken carcass in Korea. This discovery reinforces the need to understand the epidemiology of this strain. The complete genome sequence of this strain is expected to provide the foundation for further comparative genomic studies addressing the evolution and dissemination of this strain in poultry.

GenBank accession number

The complete genome sequence has been deposited in GenBank with accession no. **CP037917**.

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Competing interests

None declared.

Ethical approval

Not required.

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