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Genome Note

The first report on genome sequence of high-level ciprofloxacin-resistant *Salmonella enterica* serovar Indiana ST17 in Korean livestock

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ABSTRACT

Objectives: *Salmonella enterica* Indiana ST17 strain (K16SI097), exclusively found in China, was first isolated in 2016 in Korea from a chicken carcass. This strain contains multidrug-resistant genes, and is particularly resistant to ciprofloxacin (64 μg/mL). In this study, we aimed to elucidate the genomic relationship and compare antimicrobial resistance factors between Korean chicken-derived and Chinese clones of *S.* Indiana ST17.

Methods: The genomic DNA of *S. enterica* Indiana K16SI097 was sequenced via the combined analysis of 20-kb PacBio SMRTbell library and PacBio RS II. Antimicrobial resistance genes were analysed by the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Chromosomal and plasmid DNA of the Korean and Chinese strains were compared.

Results: The K16SI097 genome comprises two contiguous sequences (contigs) amounting to 4 731 335 base pairs with a 51.85% GC content. In total, 4574 protein-coding regions, 84 tRNA genes, and 22 rRNA genes were detected. Among the annotated contigs, 14 antimicrobial resistance genes were detected; DNA gyrase and topoisomerase IV contained mutations. Moreover, chromosomal DNA of K16SI097 and of the published Chinese strain displayed 99.9% similarity. Furthermore, plasmids displayed similar sizes, sequences, and structures.

Conclusions: This is the first report on the complete genome sequence of the high-level ciprofloxacin-resistant *S. enterica* Indiana ST17 strain isolated in Korea. This genome sequence will help us understand the ST17 strain lineage and its features such as antimicrobial resistance.

1. Introduction

Salmonella enterica is a major foodborne pathogen in both humans and animals. The drugs of choice for treating salmonellosis are fluoroquinolones or cephalosporins [1]. The extensive use of fluoroquinolones in livestock, including in Korea, is a concern because of the potential acquisition of resistance among microbial strains and thus affecting humans. Ciprofloxacin-resistant non-typhoidal *Salmonella* strains can infect humans and cause vomiting, diarrhoea, fever, gastroenteritis, and acute cholecystitis [1].

Recently, we detected high levels of ciprofloxacin-resistant *S.* Indiana ST17 strain in Korean livestock. However, limited information is available regarding the genome and antimicrobial resistance of the *S.* Indiana ST17 strain in Korea. This study aimed to explore the genomic characteristics such as genome basis, antimicrobial genes, and phylogenetic relationship of the *S.* Indiana ST17 strain.

2. Materials and methods

The *S. enterica* Indiana ST17 strain, designated K16SI097, was isolated from a chicken carcass in Chungcheongbuk-do Province, South Korea in 2016. After enrichment in tryptic soy agar at 37 °C (Becton Dickinson, Sparks, MD, USA), the strain was identified as belonging to the *Salmonella* serovar using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry

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(bioMérieux, Marcy l'Étoile, France) and *Salmonella* antisera (Becton, Dickinson and Company).

Minimum inhibitory concentrations (MICs) for ciprofloxacin and nalidixic acid antimicrobials were determined through agar dilution methods per CLSI (2019) guidelines.

The genomic library of the K16SI097 strain was obtained via the PacBio SMRTbell library preparation kit and PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA). De novo assembly and consensus polishing were analysed via the HGAP 2.0 package in the SMRT version 2.3.0 software. Assembly data were circularised using Circlator 1.4.0 [2]. Gene prediction and annotation were performed using NCBI Prokaryotic Genome Annotation Pipeline, Glimmer 3.02 [3] and the Clusters of Orthologous Groups (COG; [4]). Antibiotic resistance genes were screened by the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) ResFinder webtool (v3.2) with default parameters. Two chromosomes (CP041181 and CP041179) and plasmids (CP041180 and CP041182) were obtained from the Chinese *S. enterica* Indiana ST17 from the NCBI database and compared with those of K16SI097. The query genome was divided into 1020-bp fragments, and high-scoring pairs were compared between these two sequences via the unweighted pair group method. Pan-genome analysis and sequence alignments were performed using Roary v1.007 and MAFFT v7.427.

3. Initial findings

Two contigs were generated using the PacBio Systems. The genome comprised 4 731 335 bases with a total coverage of 185.62× and a 51.85% GC content. The chromosome comprised 4574 predicted protein-coding sequences, 84 tRNA genes, and 22 rRNA genes. Abundant categories in the COG distribution were amino acid and carbohydrate transport and metabolism. Multilocus sequence typing based on the *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA* genes were confirmed with the genome belonging to ST17 (allelic profile 8-8-11-11-5-11-15).

The MICs for ciprofloxacin and nalidixic acid were 64 and 128 μg/mL, respectively. The K16SI097 plasmid contained several antibiotic resistance genes including those for resistance to quinolone (*aac(6')-Ib-cr*), rifampicin (*ARR-3*), phenicol (*catA1*, *catB3*, *floR*), beta-lactam (*blaOXA-1*, *blaTEM-1B*), tetracycline (*tetA*), sulfonamide (*sul2*), and aminoglycoside (*aac(3)-IV*, *aph(3")-Ib*, *aph(4)-Ia*, and *aph(6)-Id*). An aminoglycoside resistance gene (*aac(6')-Iaa*) was detected in the K16SI097 strain chromosome. The primary mechanisms potentially underlying fluoroquinolone resistance were DNA gyrase and topoisomerase IV mutations, such as S83 F and D87 G in *gyrA* and T57S and S80R in *parC* which were identified as the China *S.* Indiana strain [5]. Upon analysis, the K16SI097 plasmid had a similar size (273 233 bp) to that of the Chinese strain (from 236 217 bp to 240 209 bp) and a similar plasmid structure, including *IS26 – qacE – ARR-3 – catB3 – OXA-1 – aac(6')-Ib-cr – IS26*.

On ANI tree analysis (Fig. 1) and pan-genome analysis (Supplement S1, S2), the K16SI097 chromosome displayed a close relationship with that of the Chinese strain, which is exclusively found in China [1]. Of the total 4530 gene clusters, 4240 (93.6%) are shared among the chromosomes of the three strains. However, the K16SI097 chromosome contained specific genes such as *chbR* (transcriptional regulator), *guaA* (glutamine-hydrolysing GMP

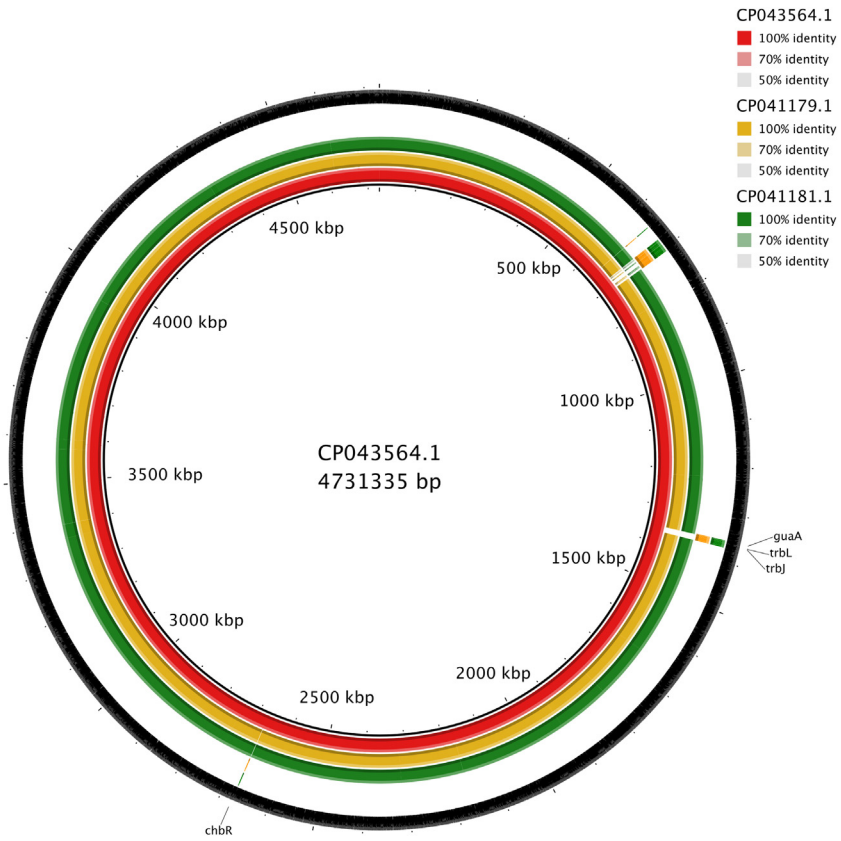

Fig. 1. Genomic relationship of *Salmonella enterica* serovar Indiana Sequence Type 17 Strain K16SI097 with two Chinese *S. enterica* Indiana genome sequences. A total of two genome sequences were obtained from *S. enterica* Indiana from the NCBI database and compared with those of strain K16SI097 by calculating average nucleotide identity values. Green circle: CP041181 (China, chicken, 20-May-2013); yellow circle: CP041179 (China, chicken, 01-Dec-2010); red circle: CP043564 (Korea, chicken, 09-Dec-2016).

synthase), *trbL* (P-type conjugative transfer protein TrbL), and *trbJ* (P-type conjugative transfer protein TrbJ).

This is the first report on the genome sequence of the high-level ciprofloxacin-resistant *S.* Indiana ST17 strain. According to Korean livestock antimicrobial resistance monitoring results, since first identified in 2016, *S.* Indiana has been continuously identified in chickens and ducks; moreover, the frequency of *S.* Indiana is increasing. Our findings provide the foundation for future studies on the pathogenicity of *S.* Indiana ST17 strains that, in turn, may provide further insight into approaches to control their spread.

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Competing interests

None declared.

Ethical approval

Not required.

GenBank accession no

The genome sequence has been deposited in GenBank with the accession no. CP043564 (chromosome), CP052938 (plasmid).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2020.12.022.

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