Complete Genome Sequence of Avian Pathogenic *Escherichia coli* Strain APEC O2-211

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ABSTRACT  Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis, a disease that affects poultry production worldwide and leads to multimillion-dollar losses annually. Here, we report the genome sequence of APEC O2-211, a sequence type 117 (ST117) strain isolated from a diseased chicken.

APEC O2-211 was stored at −80°C in glycerol prior to sequencing, and the isolate was cultured on MacConkey agar. Next, a single colony was picked and grown in Luria-Bertani broth at 37°C. DNA was extracted using a DNeasy blood and tissue Genomic-tip kit (Qiagen, Hilden, Germany) for Pacific Bioscience (PacBio, Menlo Park, CA) sequencing and a ChargeSwitch genomic DNA (gDNA) mini bacteria kit (Life Technologies, Carlsbad, CA) for Illumina sequencing. DNA yields were quantified using a Qubit fluorometer double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies). The genomic library for MiSeq sequencing was prepared with a Nextera Flex kit (Illumina, San Diego, CA), and a SMRTbell kit (Pacific Biosciences, Menlo Park, CA) was used to prepare the genomic library for PacBio sequencing with BluePippin (Sage Science, Beverly, MA) size selection to target a library size of 10 kb.

Genomic sequencing was performed on RS II (PacBio, Menlo Park, CA) and MiSeq (Illumina, San Diego, CA) instruments. Two single-molecule real-time (SMRT) cells were used for PacBio sequencing. The Illumina data were trimmed using Trimomatic v. 0.36 (4) and assembled de novo using the SPAdes assembler v. 3.10.1 (5). The PacBio data were assembled with Canu v. 1.5 (6). Next, the PacBio assembly was circularized in Geneious v. 10.2.3 (7) and further corrected and polished once with paired Illumina reads utilizing Pilon v. 1.22 (Broad Institute) (8). Potential contigs with plasmids were identified from both sets of sequencing data with the PlasmidFinder database (9) in ABRicate (https://github.com/tseemann/abricate). Protein-encoding genes and tRNA-carrying genes were assessed via Prokka v. 1.13 (10). A complete workflow and assembly report can be found at http://github.com/nielsend/genomeassembly.

The genome of APEC O2-211 consists of a single chromosome, one large plasmid, and two small plasmids. The chromosome consists of 5,114,241 bp and has a 50.6% GC content. It encodes 89 tRNAs and contains 4,783 coding sequences. The large plasmid,
pAPEC02-211A-ColV, is a hybrid IncFIB/IncFIC plasmid. It consists of 197,773 bp with 215 coding sequences and has a 49.1% GC content. The two small plasmids, pAPEC02-211B and pAPEC02-211C, consist of 4,231 and 2,096 bp, respectively. Genomic comparisons of APEC O2-211 with other extraintestinal pathogenic E. coli (ExPEC) are ongoing.

Data availability. The chromosomes and plasmids have been deposited in GenBank under the accession numbers CP006834, CP030791, CP030792, and CP030793. The PacBio and Illumina reads are available in the NCBI Sequence Read Archive (accession number SRP158042).

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REFERENCES

1. Kapur V, White DG, Wilson RA, Whittam TS. 1992. Outer membrane protein patterns mark clones of Escherichia coli O2 and O78 strains that cause avian septicaemia. Infect Immun 60:1687–1691.

2. Nielsen DW, Klimavicz JS, Cavender T, Wannemuehler Y, Barbieri NL, Nolan LK, Logue CM. 2018. The impact of media, phylogenetic classification, and E. coli pathotypes on biofilm formation in extraintestinal and commensal E. coli from humans and animals. Front Microbiol 9:902. https://doi.org/10.3389/fmicb.2018.00902.

3. Logue CM, Wannemuehler Y, Nicholson BA, Doetkott C, Barbieri NL, Nolan LK. 2017. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal Escherichia coli using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic Escherichia coli (APEC) classification. Front Microbiol 8:283. https://doi.org/10.3389/fmicb.2017.00283.

4. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pyshkin AV, Pyshkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

6. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.

7. Kears M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Genious Basic, an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

8. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakhikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.

9. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.

10. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068. https://doi.org/10.1093/bioinformatics/btu153.