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Differentiation of closely related *Salmonella enterica* serotype Heidelberg isolates by comparative genomic analysis

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Introduction: *Salmonella enterica* serovar Heidelberg is one of the top serovars responsible for numerous human outbreaks, including a 2011 multistate outbreak by an antibiotic-resistant strain. This outbreak involved 136 confirmed cases, with one death, resulting from consumption of contaminated ground turkey.

Purpose: Our objective was to explore how whole genome sequencing (WGS) can rapidly differentiate closely-related *S. Heidelberg* isolates, and provide data from which a better understanding of the evolution and ecology of S. Heidelberg can be gained.

Methods: DNA from 44 S. Heidelberg isolates collected from various sources over 30 years, including the 2011 ground turkey outbreak, was sequenced using the 454 GS FLX (Roche) platform. Phylogenetic analyses were conducted on a matrix of single nucleotide polymorphisms (SNPs) identified with the program kSNP. A DNA preparation from a clinical outbreak strain was used to synthesize a single continuous long read library that was sequenced and assembled using the Pacific Biosciences (PacBio) RS sequencer and their hierarchical genome assembly process.

Results: SNP analysis distinguished strains sharing the same PFGE patterns. The 2011 outbreak strains clustered together having only two diagnostic SNPs differences among them. Furthermore, the analysis identified a variety of antimicrobial resistance and virulence plasmids. In particular, the outbreak isolates contained a mobilizable- ColE1 plasmid, the VirB/D4 virulence plasmid that carries the type IV secretion system, and an incompatibility group (Inc) I antimicrobial resistance plasmid encoding resistance to gentamicin (*aacC2*), beta-lactam (*bl2b*_tem), streptomycin (*aadAI*) and tetracycline (*tetA*, *tetR*). Additionally, the complete, closed genome/plasmids sequence from a clinical isolate was rapidly determined using the PacBio system providing more nucleotide sequences for analyses.

Significance: This study shows that WGS can, in combination with other methods, serve as a powerful tool for separating strains considered clonal by indistinguishable PFGE profiles. The timely application of WGS technology will advance investigations to identify bacterial source and to understand the outbreak transmission dynamics of *Salmonella*.

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