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GENOME ANNOUNCEMENT

Genetic Fine Structure of a *Salmonella enterica* Serovar Typhi Strain Associated with the 2005 Outbreak of Typhoid Fever in Kelantan, Malaysia

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Among enteric pathogens, *Salmonella enterica* serovar Typhi is responsible for the largest number of food-borne outbreaks and fatalities. The ability of the pathogen to cause systemic infection for extended durations leads to a high cost of disease control. Chronic carriers play important roles in the evolution of *Salmonella* Typhi; therefore, identification and in-depth characterization of isolates from clinical cases and carriers, especially those from zones of endemicity where the pathogen has not been extensively studied, are necessary. Here, we describe the genome sequence of the highly virulent *Salmonella* Typhi strain BL196/05 isolated during the outbreak of typhoid in Kelantan, Malaysia, in 2005. The whole-genome sequence and comparative genomics of this strain should enable us to understand the virulence mechanisms and evolutionary dynamics of this pathogen in Malaysia and elsewhere.

Salmonella enterica serovar Typhi and other pathogenic salmonellae are endemic in some countries (8, 15), and outbreaks occur due to unhygienic conditions (16), leading to alarming morbidity and mortality figures (9, 18). This results in a huge burden on the public health machinery. *Salmonella* Typhi persists for an extended duration in its host (18) due to modulation of the host immune responses together with genetic rearrangements that confer fitness advantages (12, 13, 17).

The incidence of typhoid in Kelantan had always been higher than in other states of Malaysia. In the 2005 outbreak (April to June 2005), 735 cases and 2 deaths occurred (18). We hypothesized that the genome sequences of the underlying strains would provide more insights to enhance understanding of endemicity or persistence of typhoid in Kelantan.

Salmonella Typhi BL196/05 was isolated from blood samples of a severe typhoid case in Kelantan during the notorious outbreak of 2005. The strain was characterized by PCR to determine the presence of many different virulence genes. The genome sequence was analyzed and annotated exactly as described previously (4, 5, 11, 21). Briefly, the 73-bp paired-end Illumina sequence reads, amounting to 1.7 gigabytes of data (insert size, 300 bp), were generated with 80× genome coverage. Velvet (26), with the hash length set to 37, was used to assemble sequence reads into 191 contigs; these were further assembled into a draft genome and were submitted to RAST (6) to determine the following data. The size of the single chromosome was approximately 4,744,056 bp, with a G+C content of 53.21% and a coding percentage of 87.1. There were 4,875 protein coding sequences found, with an average length of 875 bp. The genome revealed 76 tRNA and 22 rRNA genes. Our strain did not harbor any plasmid, and its phage typing revealed Vi phage type B1. The genome sequence revealed two *mar* regulons, *marRAB* and *marC*, as reported also in *Salmonella* Typhi strains CT18 and Ty2, and these were homologous to *Escherichia coli mar* (multiple antibiotic resistance) regulon members (2, 3, 10, 20, 25). The genes encoding a melittin resistance protein, PqaB, and a polymyxin resistance protein, PmrD (7), were located in the genome as also seen in the *Salmonella* Typhi CT18 and Ty2 genomes. The methyl viologen resistance gene (14) *smvA* was also identified. Several pathogenicity islands as well as a pool of hypothetical proteins were annotated.

Considerable genetic diversity exists among human isolates of *Salmonella* Typhi in Malaysia and Southeast Asia (22, 23), and the heterogeneity in genome sizes (24) points to a high degree of plasticity in the *Salmonella* pan-genome. The genome sequence of strain BL196/05 also revealed rearrangements putatively relevant to virulence optimization, persistence, and adaptation within the host. Such information would be harnessed to improve understanding of genome evolution and adaptation dynamics of *Salmonella* in Malaysia and to develop point-of-care diagnostics. Further, future efforts are needed to evaluate the significance of comparative genomics/genotypic data in juxtaposition with host genetic polymorphisms to herald the beginning of the "functional molecular infection epidemiology" (1) of typhoid. Finally, we recommend greater use of data determined in studies of clinical isolates and strains specific to outbreaks and countries rather than use of the reference (type) strains alone.

Nucleotide sequence accession numbers. This genome project has been deposited at GenBank under accession no. AJGK00000000. The version described herein is the first version, AJGK01000000. The Bioproject designation for this project is PRJNA85621.

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