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GENOME SEQUENCES

Draft Genome Sequences of Antimicrobial-Resistant *Shigella* Clinical Isolates from Pakistan

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ABSTRACT *Shigella* spp. are the most common cause of dysentery in developing countries and the second leading cause of diarrheal deaths worldwide. Multidrug-resistant (MDR) *Shigella* spp. are a serious threat to global health. Herein, we report draft genome sequences for three MDR *Shigella* isolates from Pakistan, two *Shigella flexneri* isolates and one *Shigella sonnei* isolate.

Among the *Enterobacteriaceae*, the genus *Shigella* is composed of four species (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*) that cause severe diarrhea (1). Shigellosis is the most common cause of dysentery in developing countries and the second leading cause of diarrheal deaths worldwide (1). Rarely, *Shigella* spp. can also cause bacteremia, usually in children and immunocompromised adults (2, 3). The emergence of antimicrobial resistance (AMR) among *Shigella* spp. increasingly threatens the clinical management of shigellosis (4, 5). Multidrug-resistant (MDR) *Shigella* spp. are a serious threat to global health (6).

We report the draft genome sequences of three MDR *Shigella* isolates from Pakistan. The *S. flexneri* isolates were acquired from blood in 2015 (CFSAN059650) or stool (CFSAN059651 [original collection date unavailable]). The *S. sonnei* isolate (CFSAN059652) was obtained from stool in 2016. The isolates were obtained using standard culture methods, including growth on MacConkey agar, and identification and antimicrobial susceptibility testing were performed with the API 20E system and disk diffusion method (both bioMérieux), respectively (7). The results were confirmed with the Vitek 2 system (bioMérieux) and conventional broth microdilution (8). All isolates were resistant to ampicillin, cefazolin, ceftriaxone, cefotaxime, trimethoprim-sulfamethoxazole, and tetracycline. CFSAN059651 was resistant to cefepime, aztreonam, ampicillin-sulbactam, and chloramphenicol. CFSAN059650 and CFSAN059652 exhibited intermediate resistance against ciprofloxacin and ampicillin-sulbactam or against aztreonam and ceftazidime, respectively. Isolates were grown overnight in lysogeny broth (Lennox) at 37°C prior to DNA extraction using the DNeasy blood and tissue kit (Qiagen). DNA libraries were prepared via the Nextera XT DNA library kit (Illumina). Sequencing was performed on an Illumina MiSeq platform using the MiSeq reagent kit v2 (2 × 250-bp paired-end reads). Minimum average coverage (>50×) and read 1 (R1)/read 2 (R2) Q scores (>26) were set to ensure sequence quality. Sequence integrity (i.e., absence of contamination) was evaluated with Kraken (9, 10). Unless otherwise noted, default parameters were used in all analyses. *De novo* genome

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TABLE 1 Assembly statistics and accession numbers for the reported *Shigella flexneri* and *Shigella sonnei* genome sequences[a]

Species and CFSAN ID[b]	NCBI BioSample accession no.	Yr of isolation	No. of contigs	Total length (bp)	N_{50} (bp)	GC content (%)	Genome coverage (×)	Coverage read count	Avg read quality (Q score [R1, R2])	SRR accession no.	WGS accession no.[c]
Shigella flexneri											
CFSAN059650	SAMN10086692	2015	356	4,629,951	33,749	50.55	52	1,021,504	32, 26.6	SRR8836963	SSML00000000
CFSAN059651	SAMN10086664	NA[d]	359	4,620,515	31,916	50.45	69	1,373,334	32, 27	SRR8836950	SSMK00000000
Shigella sonnei											
CFSAN059652	SAMN10086663	2016	384	4,528,736	25,093	50.85	71	1,388,868	32, 26.9	SRR8837012	SSMJ00000000

[a] All three clinical isolates belong to NCBI BioProject number PRJNA342326.
[b] ID, identifier.
[c] WGS, whole-genome sequencing.
[d] NA, not available.

assemblies were created with Shovill 0.9 (https://github.com/tseemann/shovill), available in GalaxyTrakr (https://www.galaxytrakr.org) (11). The "trim reads" option was selected, and the minimum contig length was set at 500 bp. The draft genomes were submitted for annotation to the NCBI Prokaryotic Genome Annotation Pipeline (12). The number of reads, number of contigs, genome size, and GC content for each isolate are listed in Table 1.

ResFinder (13) was used to determine isolate sequence types (STs) based on *in silico* multilocus sequence typing (MLST) (14) and to identify AMR genes showing >99% identity to the reference gene sequences. The two *S. flexneri* isolates belonged to ST245, and the *S. sonnei* isolate belonged to ST152. CFSAN059650, CFSAN059651, and CFSAN059652 were found to harbor 10, 8, and 7 known AMR genes, respectively. All *Shigella* isolates carried *drfA1*, *sul2,* a CTX-M gene, and a *tet* gene. All isolates possessed AMR genes conferring resistance to β-lactams, aminoglycosides, trimethoprim, sulfonamides, and tetracyclines. Additional genes involved in resistance to fluoroquinolones (CFSAN059650 and CFSAN059652) or phenicol (CFSAN059651) were also observed. The data provided here offer a comparative genetic context for AMR in *Shigella* spp. that will inform infectious diseases epidemiology and be useful in public health monitoring.

Data availability. The genome assemblies described herein are available in DDBJ/EMBL/GenBank under accession numbers SSMJ00000000, SSMK00000000, and SSML00000000. The versions here are the first versions. Other accession number data are presented in Table 1.

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