



# Genome Sequencing Analysis of Atypical *Shigella flexneri* Isolated in Korea

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**Objectives:** An atypical *Shigella flexneri* strain with a plural agglutination pattern [i.e., reacting not only with serum samples containing type antigen II but also with serum samples containing group antigens (3)4 and 7(8)] was selected for genome sequencing, with the aim of obtaining additional comparative information about such strains.

**Methods:** The genomic DNA of atypical *S. flexneri* strain NCCP 15744 was sequenced using an Ion Torrent PGM sequencing machine (Life Technologies, USA). The raw sequence data were preprocessed and reference-assembled in the CLC Assembly Cell software (version 4.0.6; CLC bio, USA).

**Results:** Ion Torrent sequencing produced 1,450,025 single reads with an average length of 144 bp, totaling ~209 Mbp. The NCCP 15744 genome is composed of one chromosome and four plasmids and contains a *gtrX* gene. Among the published genome sequences of *S. flexneri* strains, including 2457T, Sf301, and 2002017, strain NCCP 15744 showed high similarity with strain 2002017. The differences between NCCP 15744 and 2002017 are as follows: i) NCCP 15744 carries four plasmids whereas 2002017 carries five; ii) 19 genes (including *CI*, *CII*, and *cro*) were lost in the *SHI-O* genomic island of NCCP 15744 and six genes were gained as compared with strain 2002017.

**Conclusion:** Strain NCCP 15744 is genetically similar to 2002017, but these two strains have different multilocus sequence types and serotypes. The exact reason is unclear, but the 19 lost genes may be responsible for the atypical seroconversion of strain NCCP 15744.

**Key Words:** *Shigella*, genomics, sequence analysis

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## INTRODUCTION

*Shigella* spp. are transmitted via the fecal-oral route and cause diseases by invading the colonic epithelium, which results in tissue destruction and massive inflammation. For this reason, *Shigella* spp. are causative agents of bacillary dysentery in humans [1]. Several groups of serotypically atypical *Shigella flexneri* strains were isolated in Korea in 2008. Among these, one group displayed a plural agglutination pattern by reacting with serum samples containing type antigen II as well as reacting with serum samples containing group antigens (3)4 and 7(8). This atypical *S. flexneri* strain NCCP 15744 was registered at the National Culture Collection for Pathogens (NCCP) of Korea National Institute of Health (Korea NIH) as NCCP No. 15744.

NCCP 15744 shows higher antibiotic resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole than do typical *S. flexneri* strains [2,3]. Atypical strains, or newer subserotypes, are being isolated in different parts of the world, e.g., serotype 4c, which was isolated in China and East-Asian countries [4–7]. Serotype 1c has also been reported in Bangladesh, China, Egypt, and Pakistan [8–12]. Moreover, a serotype X variant (2002017) was re-



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ported, and its genome was sequenced in China [13]. All *S. flexneri* serotypes, except serotype 6, share the same backbone of the basic O-antigen repeat unit, which is a tetrasaccharide consisting of a single *N*-acetylglucosamine and three rhamnose residues [14]. Glycosylation of any of the four sugars and/or O-acetylation of the last rhamnose residue give rise to more than 13 known serotypes. The enzymes of both processes are encoded by genes carried by bacteriophages. *N*-Glycosylation involves three Glucosyltransferases (*gtr*) genes, with one being type specific, whereas O-acetylation involves only one gene: O-acetyltransferase (*oac*) [14]. This O-antigenic variation is a major strategy used by this organism to evade the host's immunity. These bacteriophage-encoded modifications allow *S. flexneri* to change its O-antigenicity rather simply.

In this study, we analyzed the genome profile of NCCP 15744 and compared it with that of 2002017 as a reference strain. Strain 2002017 was a dominant serotype in China from 2003 to 2006 and was identified as a variant of serotype X. NCCP 15744 emerged for the first time in Korea in 2008, with less than 10 isolated strains per year. (i.e., 2011: 6 strains, 2012: 7 strains, 2013: 1 strain, 2014: 1 strain, and 2015: 1 strain). The major serotype of *S. flexneri* in Korea was 2a in the same period.

It would be worthwhile to characterize atypical *S. flexneri* in relation to infections. Additionally, the atypical *S. flexneri* strains were isolated from children and adults with severe dysentery; these data highlight the need to study these isolates in detail. Specifically, the atypical *S. flexneri* presented in this study should also be subjected to analysis and identification of novel O-antigen modification genes. Therefore, it would be useful to analyze the genome by sequencing for comparison with the known genomes of other strains.

## MATERIALS AND METHODS

### 1. Sample collection and biochemical characterization

The atypical *S. flexneri* NCCP 15744 strain was isolated from a 2-year-old diarrheal patient in 2008. This strain was identified as *S. flexneri* by means of the API 20E kit (bioMérieux, Marcy-l'Étoile, France). The serotype of the strain was confirmed using a commercially available antiserum kit (Denka Seiken, Niigata,

Japan) specific for all type and group factor antigens. Serological reactions were run according to the manufacturer's instructions.

### 2. Polymerase chain reaction (PCR) and multilocus sequence type (MLST) analysis

Chromosomal DNA was purified using the Genomic-Prep DNA Isolation Kit (Amersham Biosciences, Roosendaal, Netherlands). All PCRs were conducted using the Expanded High Fidelity Polymerase System (Roche Diagnostics, Mannheim, Germany) or *Taq* polymerase (Takara Bio Inc., Shiga, Japan). The primers used for *gtr* and *oac* gene analysis are listed in **Supplementary Table S1**. The PCR product was purified and sequenced. For MLST analysis, seven housekeeping genes (i.e., *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were amplified by PCR and sequenced. This analysis was conducted according to a previously published report and MLST web site (<http://mlst.ucc.ie/>) [15].

### 3. Genome sequencing

Genomic DNA was isolated from the atypical *S. flexneri* NCCP 15744 strain using standard protocols and was sequenced on an Ion Torrent PGM sequencing machine (Life Technologies, Carlsbad, CA, USA). The Ion Torrent sequencing produced 1,450,025 single reads with an average length of 144 bp, totaling ~209 Mbp.

### 4. Sequencing data analysis

Raw sequence data were preprocessed and reference-assembled using the CLC Assembly Cell software (version 4.0.6; CLC bio, Waltham, MA, USA). Eight novel genes were predicted in the de novo contig, using Glimmer 2.1 [16] with default options. Genomic localization of insertion elements was assessed using ISfinder [17], filtered with an e-value cutoff of 1E-5 and subject coverage of 50%. To compare the overall chromosomal organization among four strains (i.e., NCCP 15744, 2002017, sf301, and 2457T), dot-plots between the reference genomes were first generated using MUMmer [18]. Additionally, the colinear blocks between the genomes were analyzed by means of the Mauve algorithm [19], and the result was parsed and visualized within a circular map by means of Circos (<http://circos.ca/>). The average nucleotide identity (ANI) values were calculated in the JSpecies software and aligned by the Basic Local Alignment Search Tool

**Table 1.** Average of nucleotide identities (ANI) of strain NCCP 15744 compared to 3 reference strains (2002017, Sf301, and 2457T)

Target	Query	ANiB	ANiB alignments	ANiB aligned
2002017	NCCP 15744	99.91%	4,557 (95.49%)	4,642,181 (95.36%)
Sf301	NCCP 15744	99.83%	4,446 (93.17%)	4,519,560 (92.83%)
2457T	NCCP 15744	99.84%	2,264 (93.55%)	4,540,984 (93.20%)

(BLASTn) [20,21] (Table 1). A prophage search was conducted by means of the PHAge Search Tool (PHAST; <http://phast.wis-hartlab.com>) [22].

### 5. The nucleotide sequence accession number

The National Center for Biotechnology Information (NCBI) accession number for the genome sequence of atypical *S. flexneri* NCCP 15744 reported in this study is AWOX00000000.

## RESULTS

### 1. Sequencing data analysis

Raw sequence reads were generated by Ion Torrent PGM sequencing. The 648,242 reads (~96 Mbp, average length 148 bp) were assembled using the genome of strain 2002017 (serotype X variant; gbAcc. NC\_017328) as a reference. As a result, 620,506 reads (95.7%) were successfully aligned with the reference genome. By combining the reference-assembled contigs with the single de novo contig, a pseudomolecule of genome size 4,641,722 bp was generated for the NCCP 15744 strain (Table 1).

### 2. Genome features

Sequence reads were mapped to the published genomes of three reference strains (i.e., 2457T, Sf301, and 2002017), resulting in reads that were best aligned with the 2002017 genome (Table 1). The NCCP 15744 genome was thus found to be composed of one chromosome and four plasmids (pSF<sub>II-1</sub>, pSF<sub>II-2</sub>, pSF<sub>II-3</sub>, and pSF<sub>II-4</sub>), including a large virulence plasmid and a drug resistance plasmid (pSF<sub>II-1</sub>; Table 2). The chromosome sizes of NCCP 15744 and 2002017 are similar (4,631,995 bp in NCCP 15744 vs. 4,650,865 bp in 2002017), but strain 2002017 has five plasmids instead of four.

### 3. Seroconversion-related genes (SHI-O) and antibiotic resistance-related genes (SRL and SRLII)

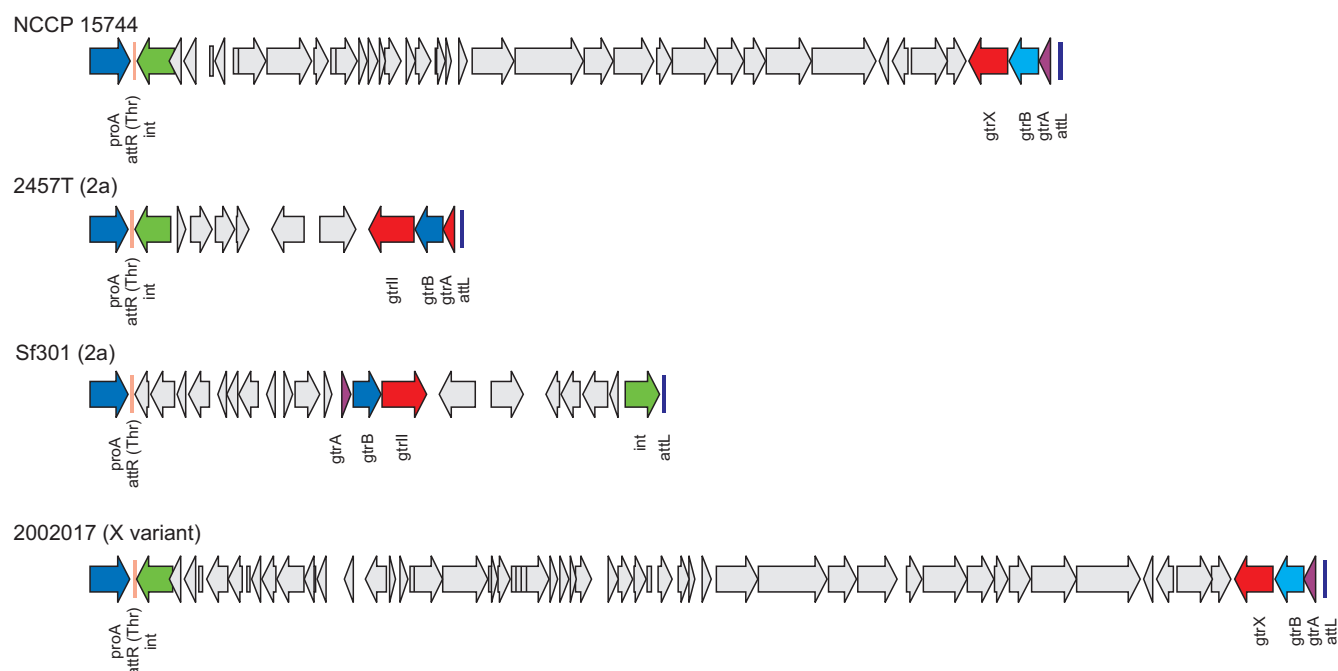
SHI-O (37,894 bp) is the serotype conversion island in *S. flexneri*, carrying genes for O-antigen modification. The SHI-O island of NCCP 15744 is located at the same site as are SHI-O genes of other *S. flexneri* reported previously [23]. As confirmed by PCR analysis, this site contains *gtrA*, *gtrB*, and *gtrX* genes for serotype X, 2b, and 3b conversion, identical to the SfX *gtr* genes published elsewhere. Nevertheless, 19 genes were lost in strain NCCP 15744 compared with strain 2002017 [24,25] (Figure 1, Table 3).

The 48,095-bp *Shigella* resistance locus (SRL) contains tetracycline, chloramphenicol, ampicillin, and streptomycin resistance genes. It is similar to the SRL island initially discovered in the *S.*

Table 2. Genome features of *Shigella flexneri* NCCP 15744 and 2002017

	Features of NCCP 15744					Features of 2002017				
	Chromosome	Plasmids				Chromosome	Plasmids			
	pSF <sub>II-1</sub>	pSF <sub>II-2</sub>	pSF <sub>II-3</sub>	pSF <sub>II-4</sub>		pSF <sub>XV-1</sub>	pSF <sub>XV-2</sub>	pSF <sub>XV-3</sub>	pSF <sub>XV-4</sub>	pSF <sub>XV-5</sub>
Total length (bp)	4,631,995	223,049	293	4,268	4,650,865	223,364	6,850	6,200	4,042	3,180
No. of ORFs	79.6	75.2	45.92	50.86	83.3	79.3	64.2	71.5	58.9	61.8
Percentage of CDS (%)	336 (7)	216	13	98	491 (6)	157 (32)	1	0	0	0
G+C content (%)	216	13	98	98	50.86	45.92	45.92	45.92	52.55	45.4
IS elements (%)	216	13	98	98	491 (6)	157 (32)	1	0	0	0
No. of pseudogenes	216	13	98	98	232					
No. of rRNAs	13	13	98	98	22					
No. of tRNAs	98	98	98	98	101					

ORF, open reading frame; CDS, coding sequence; IS, insertion sequence



**Figure 1.** Alignment of *SHI-O* loci of strains NCCP 15744, 2457T, Sf301, and 2002017.

**Table 3.** The list of genes lost in strain NCCP 15744 compared to strain 2002017 in the *SHI-O* genomic island

No.	Locus_tag	Gene product	Function
1	SF <sub>XV</sub> _0311	Hypothetical protein	Unknown
2	SF <sub>XV</sub> _0312	Putative bacteriophage protein	
3	SF <sub>XV</sub> _0313	Hypothetical protein	
4	SF <sub>XV</sub> _0315	Hypothetical protein	
5	SF <sub>XV</sub> _0316	Hypothetical protein	
6	SF <sub>XV</sub> _0317	Putative phage-related DNA recombination protein	
7	SF <sub>XV</sub> _0318	Hypothetical protein	
8	SF <sub>XV</sub> _0322	Antitermination of transcription at nut site protein	Immunity and regulation
9	SF <sub>XV</sub> _0323	CI protein	
10	SF <sub>XV</sub> _0323a	cro protein	
11	SF <sub>XV</sub> _0324	Putative regulatory protein CII of bacteriophage	
12	SF <sub>XV</sub> _0327a	Gp56	DNA replication and recombination
13	SF <sub>XV</sub> _0327b	Gp60	
14	SF <sub>XV</sub> _0328a	NinD protein	
15	SF <sub>XV</sub> _0328b	NinE protein	
16	SF <sub>XV</sub> _0335	Rz protein	Lysis
17	SF <sub>XV</sub> _0335a	Hypothetical protein	
18	SF <sub>XV</sub> _0335b	Hypothetical protein	
19	SF <sub>XV</sub> _0347	Gene 10 protein	DNA packaging & head and tail morphogenesis

*flexneri* strains 2a and YSH6000 [26,27]. *SRLII* (14,067 bp) contains multiple antibiotic resistance genes, including dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (*sat1*), and aminoglycoside adenylyltransferase (*aadA1*), which confer resistance to trimethoprim and streptomycin/spectinomycin, respectively [28,29]. The NCCP 15744 genomic islands *SRL* and *SRLII* were found to be almost identical to those of strain 2002017.

#### 4. Lost and gained genes

In total, 132 genes were lost (112 partially and 20 completely) in NCCP 15744 compared with 2002017. Among these lost genes, 19 are missing in the *SHI-O* genomic island region; seven have an unknown function, four are in the functional category of immunity and regulation, four are involved in DNA replication and recombination, three in lysis, and one gene is related to DNA packaging (Table 3). Meanwhile, on the basis of the de novo contig, six genes were predicted to be gained in NCCP 15744 compared with 2002017, most of which have an unknown function (Supplementary Table S2).

#### 5. PCR and MLST analysis

PCR analysis against serotype-specific *gtr* (including *gtrII*, *gtrV*, and *gtrX*) and *oac* was carried out. Strain NCCP 15744 was found to carry *gtrX*, but not *gtrII*, *gtrV*, or *oac*. The MLST results indicated that NCCP 15744 belongs to the sequence type complex 245 (which has the allele profile 6, 61, 6, 11, 13, 3, and 50 in the order *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*, respectively). ST245 is a well-known sequence type in Asian countries and is different from that of 2002017 (ST91).

## DISCUSSION

Genome sequencing of strain NCCP 15744 revealed that it bears four plasmids and has acquired a *Shigella* serotype conversion island (*SHI-O*), via bacteriophage SfX (*gtrX*), which is responsible for the group 7(8) antigenic determinant. SfX (*gtrX*) converts serotype Y to serotype X, Y to 3b, and 2a to 2b [30].

The NCCP 15744 genome shares the highest similarity with that of 2002017, but some differences exist between these strains. First, NCCP 15744 has only four plasmids as opposed to five in 2002017 (plasmid pSF<sub>XV\_2</sub> is absent in NCCP 15744). Second, according to MLST, 2002017 is ST91 whereas NCCP 15744 is ST245. Third, in comparison with strain 2002017, 19 genes were lost in *SHI-O* and six novel genes were gained in NCCP 15744. Among these lost genes, three (*CI*, *CII*, and *Cro* repressor) in the functional category of immunity and regulation are the regulatory switches that determine whether the Sf bacteriophage would

follow a lytic or lysogenic cycle [31,32].

The genomic sequence data revealed that the NCCP 15744 strain has gained two multi-antibiotic resistance genomic islands (*SRL* and *SRLII*) encoding genes that confer resistance to five antibiotics (i.e., tetracycline, streptomycin, chloramphenicol, ampicillin, and trimethoprim) that are commonly used for the treatment of shigellosis in Korea. Strain NCCP 15744 even showed higher resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole than did the typical *S. flexneri* 2a strain. Unlike strain 2002012, which was a major serotype from 2003 to 2006 in China, strain NCCP 15744 was not a dominant serotype from 2003 to 2006 in Korea.

Recently, a novel *S. flexneri* O-antigen modification, addition of phosphoethanolamine to Rha<sup>II</sup> was identified [33]. The strain in question has O-antigen phosphoethanolamine transferase gene *opt* (formerly called *lpt-O*) carried by a pSF<sub>XV\_2</sub>-like plasmid. This *opt* gene inactivates the serotype-specific gene *gtrX*, which generates the Xv serotype. The pSF<sub>XV\_2</sub>-like plasmid is absent in strain NCCP 15744.

ST245 is a common sequence type in Asian countries and comprises a wide range of serotypes (i.e., 1b, 2a, 3a, 3b, 3c, 4a, 4b, 5, X, Y, and 6) [34]. In addition, in our previous study, NCCP 15744 showed 81.3% similarity with the typical 2a strain in pulsed-field gel electrophoresis analysis [2]. According to these data, NCCP 15744 is close to serotype 2a [II: (3)4] and has similarities with 2b [II: 7(8)]. This kind of atypical pattern can happen via inactivation of the *gtr* locus, resulting in reversion to either the parental or intermediate serotype [34]. Nonetheless, inactivation of the *gtr* locus was not detected in NCCP 15744. The exact reason why NCCP 15744 shows a plural agglutination pattern is still unknown. A possible explanation is actions of an unstable bacteriophage causing a loss of 19 genes (including *CI*, *CII*, and *Cro* repressor genes).

These results will facilitate functional studies of intracellularly regulated genes that may be important for the adaptation and growth strategies of this atypical *S. flexneri* strain during infection.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Labrecq EH, Schneider H, Magnani TJ, et al. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. *J Bacteriol* 1964;88:1503-18.
2. Hong S, Choi YH, Choo YA, et al. Genetic characterization of atypical *Shigella flexneri* isolated in Korea. *J Microbiol Biotechnol* 2010;20:1457-62. <https://doi.org/10.4014/jmb.1005.05019>
3. Dorman CJ, Porter ME. The *Shigella* virulence gene regulatory cascade: a paradigm of bacterial gene control mechanisms. *Mol Microbiol* 1998;29:677-84. <https://doi.org/10.1046/j.1365-2958.1998.00902.x>
4. Levine MM, Kotloff KL, Barry EM, et al. Clinical trials of *Shigella* vaccines: two steps forward and one step back on a long, hard road. *Nat Rev Microbiol* 2007;5:540-53. <https://doi.org/10.1038/nrmicro1662>
5. Pryamukhina NS, Khomenko NA. Suggestion to supplement *Shigella flexneri* classification scheme with the subserovar *Shigella flexneri* 4c: phenotypic characteristics of strains. *J Clin Microbiol* 1988;26:1147-9.
6. Pu XY, Pan JC, Wang HQ, et al. Characterization of fluoroquinolone-resistant *Shigella flexneri* in Hangzhou area of China. *J Antimicrob Chemother* 2009;63:917-20. <https://doi.org/10.1093/jac/dkp087>
7. Zhang W, Pan JC, Meng DM, et al. [PFGE of *Shigella flexneri* 4c isolates from food-poisoning outbreaks and sporadic diarrhea patients]. *Zhonghua Yu Fang Yi Xue Za Zhi* 2007;41:50-3. In Chinese. <https://doi.org/10.3760/j.issn:0253-9624.2007.01.013>
8. Stagg RM, Tang SS, Carlin NI, et al. A novel glucosyltransferase involved in O-antigen modification of *Shigella flexneri* serotype 1c. *J Bacteriol* 2009;191:6612-7. <https://doi.org/10.1128/JB.00628-09>
9. Talukder KA, Islam Z, Islam MA, et al. Phenotypic and genotypic characterization of provisional serotype *Shigella flexneri* 1c and clonal relationships with 1a and 1b strains isolated in Bangladesh. *J Clin Microbiol* 2003;41:110-7. <https://doi.org/10.1128/JCM.41.1.110-117.2003>
10. Ahmed SF, Klena J, Husain T, et al. Genetic characterization of antimicrobial resistance of *Shigella flexneri* 1c isolates from patients in Egypt and Pakistan. *Ann Clin Microbiol Antimicrob* 2013;12:9. <https://doi.org/10.1186/1476-0711-12-9>
11. Ahmed SF, Riddle MS, Wierzbza TF, et al. Epidemiology and genetic characterization of *Shigella flexneri* strains isolated from three paediatric populations in Egypt (2000-2004). *Epidemiol Infect* 2006;134:1237-48. <https://doi.org/10.1017/S095026880600642X>
12. El-Gendy A, El-Ghorab N, Lane EM, et al. Identification of *Shigella flexneri* subserotype 1c in rural Egypt. *J Clin Microbiol* 1999;37:873-4.
13. Ye C, Lan R, Xia S, et al. Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of *Shigella flexneri*. *J Clin Microbiol* 2010;48:419-26. <https://doi.org/10.1128/JCM.00614-09>
14. Allison GE, Verma NK. Serotype-converting bacteriophages and O-antigen modification in *Shigella flexneri*. *Trends Microbiol* 2000;8:17-23. [https://doi.org/10.1016/S0966-842X\(99\)01646-7](https://doi.org/10.1016/S0966-842X(99)01646-7)
15. Choi SY, Jeon YS, Lee JH, et al. Multilocus sequence typing analysis of *Shigella flexneri* isolates collected in Asian countries. *J Med Microbiol* 2007;56:1460-6. <https://doi.org/10.1099/jmm.0.47322-0>
16. Delcher AL, Harmon D, Kasif S, et al. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res* 1999;27:4636-41.
17. Siguier P, Perochon J, Lestrade L, et al. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006;34:D32-6. <https://doi.org/10.1093/nar/gkj014>
18. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 2010;5:e11147. <https://doi.org/10.1371/journal.pone.0011147>
19. Kurtz S, Phillippy A, Delcher AL, et al. Versatile and open software for comparing large genomes. *Genome Biol* 2004;5:R12. <https://doi.org/10.1186/gb-2004-5-2-r12>
20. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126-31. <https://doi.org/10.1073/pnas.0906412106>
21. Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389-402.
22. Zhou Y, Liang Y, Lynch KH, et al. PHAST: a fast phage search tool. *Nucleic Acids Res* 2011;39(Suppl 2):W347-52. <https://doi.org/10.1093/nar/gkr485>
23. Ingersoll M, Groisman EA, Zychlinsky A. Pathogenicity islands of *Shigella*. *Curr Top Microbiol Immunol* 2002;264:49-65. [https://doi.org/10.1007/978-3-642-56031-6\\_4](https://doi.org/10.1007/978-3-642-56031-6_4)
24. Guan S, Bastin DA, Verma NK. Functional analysis of the O antigen glucosylation gene cluster of *Shigella flexneri* bacteriophage SFX. *Microbiology* 1999;145:1263-73. <https://doi.org/10.1099/13500872-145-5-1263>
25. Verma NK, Verma DJ, Huan PT, et al. Cloning and sequencing of the glucosyl transferase-encoding gene from converting bacteriophage X (SFX) of *Shigella flexneri*. *Gene* 1993;129:99-101. [https://doi.org/10.1016/0378-1119\(93\)90702-5](https://doi.org/10.1016/0378-1119(93)90702-5)
26. Turner SA, Luck SN, Sakellaris H, et al. Nested deletions of the SRL pathogenicity island of *Shigella flexneri* 2a. *J Bacteriol* 2001;183:5535-43. <https://doi.org/10.1128/JB.183.19.5535-5543.2001>
27. Turner SA, Luck SN, Sakellaris H, et al. Molecular epidemiology of the SRL pathogenicity island. *Antimicrob Agents Chemother* 2003;47:727-34. <https://doi.org/10.1128/AAC.47.2.727-734.2003>
28. Lichtenstein C, Brenner S. Unique insertion site of Tn7 in the *E. coli* chromosome. *Nature* 1982;297:601-3. <https://doi.org/10.1038/297601a0>
29. Sundström L, Sköld O. The dhfrI trimethoprim resistance gene of Tn7 can be found at specific sites in other genetic surroundings. *Antimicrob Agents Chemother* 1990;34:642-50. <https://doi.org/10.1128/AAC.34.4.642>
30. Knirel YA, Sun Q, Senchenkova SN, et al. O-antigen modifications providing antigenic diversity of *Shigella flexneri* and underlying genetic mechanisms. *Biochemistry (Mosc)* 2015;80:901-14. <https://doi.org/10.1134/S0006297915070093>

31. Mehta P, Casjens S, Krishnaswamy S. Analysis of the lambdoid prophage element  $\epsilon 14$  in the *E. coli* K-12 genome. *BMC Microbiol.* 2004;4:4. <https://doi.org/10.1186/1471-2180-4-4>
32. Casjens S, Winn-Stapley DA, Gilcrease EB, et al. The chromosome of *Shigella flexneri* bacteriophage Sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. *J Mol Biol* 2004;339:379-94. <https://doi.org/10.1016/j.jmb.2004.03.068>
33. Sun Q, Lan R, Wang J, et al. Identification and characterization of a novel *Shigella flexneri* serotype Yv in China. *PLoS One* 2013;8:e70238. <https://doi.org/10.1371/journal.pone.0070238>
34. Choi SY, Jeon YS, Lee JH, et al. Multilocus sequence typing analysis of *Shigella flexneri* isolates collected in Asian countries. *J Med Microbiol* 2007;56:1460-6. <https://doi.org/10.1099/jmm.0.47322-0>