RES-063

Emergence of Multidrug-resistant and Extended-spectrum β-lactamase-producing *Shigella flexneri* 2a Clinical Isolates in Japan

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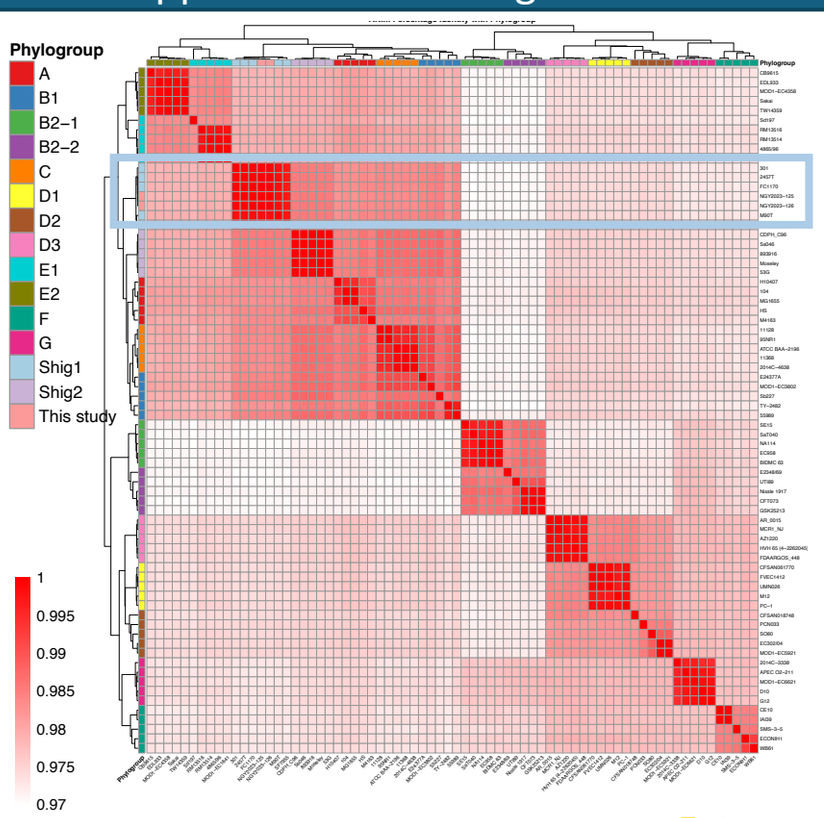
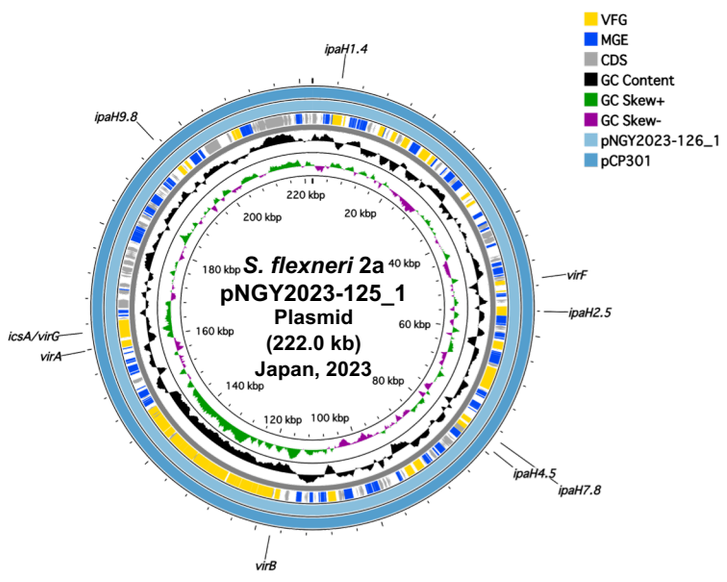
Introduction

In recent years, the emergence of fluoroquinolone-resistant and third-generation cephalosporin (3GC)-resistant *Shigella* spp. has become increasingly alarming, with incidence rates continuing to rise. The proportion of multidrug-resistant (MDR) *Shigella* spp. isolates, simultaneously resistant to fluoroquinolones such as ciprofloxacin (CIP), 3GCs such as ceftriaxone (CRO), and macrolides, particularly azithromycin (AZM), has markedly increased. A similar trend in the prevalence of MDR *Shigella* spp. has also been observed globally, predominantly in *S. sonnei*, although MDR has also been reported in *S. flexneri*, where resistance rates tend to be lower. Reflecting this growing threat, fluoroquinolone-resistant *Shigella* spp. and 3GC-resistant *Enterobacterales* have been included in the WHO Bacterial Priority Pathogen List 2024. We isolated two *S. flexneri* strains, NGY2023-125 and NGY2023-126, from family members who had each travelled to Myanmar and were treated at a medical institution in Japan in 2023. For detailed analysis, we constructed complete genome sequences using both short- and long-read sequencing technologies. In this study, we report for the first time the antimicrobial resistance profiles and genomic characteristics of MDR and ESBL-producing *S. flexneri* 2a isolated in Japan.

Result-1. Phylogenetic Analysis of *Escherichia coli* and *Shigella* spp. Based on Average Nucleotide Identity (ANI)



Biochemical testing, serotyping, and average nucleotide Identity (ANI) analysis with representative strains among *Escherichia coli* and *Shigella* spp. confirmed both isolates as *S. flexneri* serotype 2a (**phylogroup Shig1**)

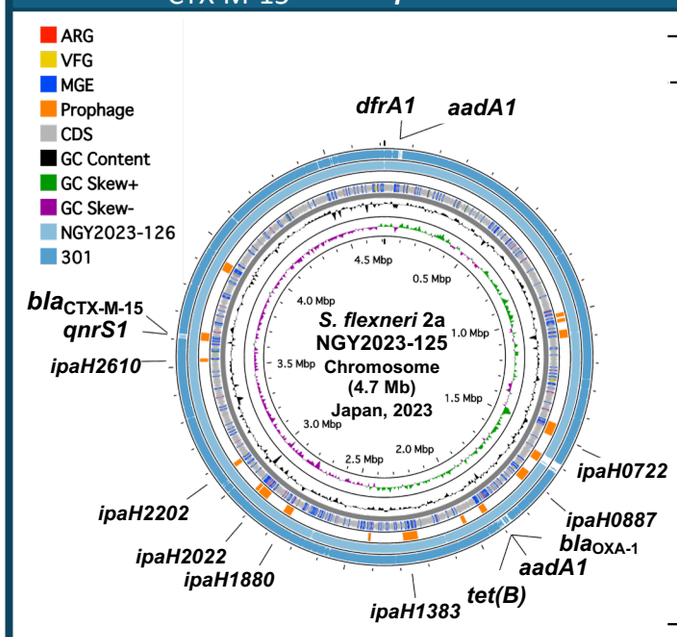


Both isolates carried multiple pathogenicity islands, including the type III secretion system (T3SS)-associated pathogenicity island, as well as virulence factor genes, such as T3SS effector genes and the *icsA/virG* gene.

Result-2. Result of Antimicrobial Susceptibility Test of The Isolates in This Study

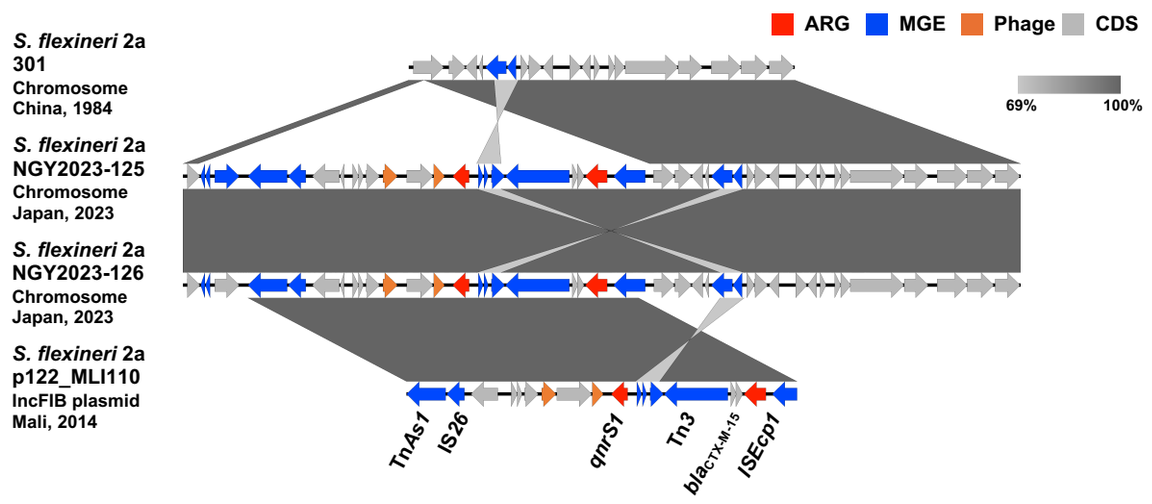
Antimicrobial agent	Method	CLSI M100-ED35 Breakpoint (R)	NGY2023-125		NGY2023-126	
			MIC / Zone	Interpretation	MIC / Zone	Interpretation
Ampicillin (AMP)	Broth microdilution	≥ 32 mg/L	>16	R	>16	R
Ceftriaxone (CRO)	Broth microdilution	≥ 4 mg/L	>32	R	>32	R
Imipenem (IPM)	Broth microdilution	≥ 4 mg/L	<0.25	S	<0.25	S
Meropenem (MEM)	Broth microdilution	≥ 4 mg/L	<0.25	S	<0.25	S
Azithromycin (AZM)	ETEST	≥ 32 mg/L	1.5	S	>256	R
Minocycline (MIN)	Broth microdilution	≥ 16 mg/L	<2	S	4	S
Ciprofloxacin (CIP)	ETEST	≥ 1 mg/L	0.25	S	0.5	I
Levofloxacin (LVX)	Broth microdilution	≥ 2 mg/L	1	I	1	I
Trimethoprim-sulfamethoxazole (SXT)	Broth microdilution	≥ 4/76 mg/L	>2/38	R	>2/38	R
Chloramphenicol (CHL)	Disk diffusion (30 µg)	≤ 12 mm (zone)	25.2	S	25.8	S

Result-3. Prophage-Mediated Chromosomal Localization of $bla_{CTX\text{-}M\text{-}15}$ and *qnrS1* Genes in ESBL-Producing *Shigella*



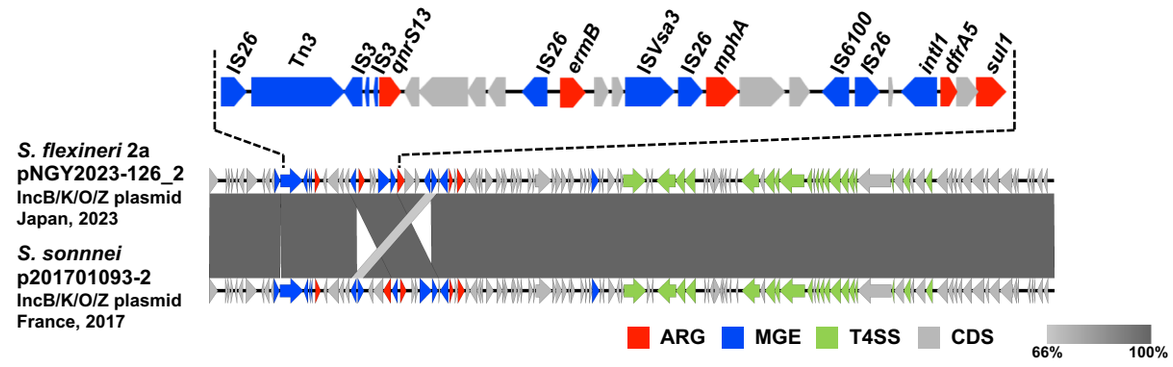
	NGY2023-125	NGY2023-126
Total Length (bp)	4,950,779 bp	5,014,783 bp
No. of Sequences	5	6
GC Content	50.6%	50.7%
N50	4,715,328	4,669,723
Gap Ratio	0%	0%
No. of CDSs	5,177	5,287
No. of rRNA	22	22
No. of tRNA	106	107
No. of CRISPRs	0	0
Coding Ratio	85.6%	86.7%
MLST	ST245	ST245

SNV analysis identified 37 differences between the two isolates, confirming they are distinct clones. NGY2023-126 carried an additional 102-kb IncB/K/O/Z-type plasmid absent in NGY2023-125. Both isolates harbored multiple ARGs, including $bla_{CTX\text{-}M\text{-}15}$, *qnrS1*, $bla_{OXA\text{-}1}$, *aadA1*, *dfrA1*, and *tet(B)*. Notably, $bla_{CTX\text{-}M\text{-}15}$ and *qnrS1* were located within a 33.4-kb prophage region containing mobile genetic elements.



A BLAST search of this region against the NCBI nr database showed 99.9% identity to *E. coli* plasmid isolated from a human in Mali in 2014

Result-4. Comparative Analysis of Azithromycin Resistance Plasmids Highlighting *mphA-ermB* Region



BLAST indicated 99.9% identity to *S. sonnei* isolated from a human in France in 2017. Recent reports have described the plasmid-mediated *mphA* gene as a major contributor to increasing macrolide resistance in *Shigella* spp.

Conclusion

In conclusion, this study presents a genomic epidemiological analysis of two MDR *S. flexneri* 2a isolates in Japan. Both isolates carry chromosomally integrated $bla_{CTX\text{-}M\text{-}15}$ and *qnrS1*, while one isolate additionally harbours a conjugative plasmid carrying ARGs, including *mphA* and *ermB*. We also identified the two distinct MDR *S. flexneri* isolates from different patients, raising concerns about the importation and local transmission of specific resistant clones from Southeast Asia. Given the rising prevalence of fluoroquinolone-, 3GC-, and macrolide-resistant *Shigella* spp. clones, along with the potential for further acquisition of ARGs via MGEs including plasmids and phages, alternative treatment options must be considered. Patients returning from the countries with a high prevalence of ESBL-producing *Enterobacterales* should be carefully assessed for infections with MDR *Shigella* spp. Integrating antimicrobial susceptibility data with genomic information, including antimicrobial resistance genotypes, is important for guiding appropriate treatment strategies.