

**Table S3. Bacterial strains used in this study**

Species	Strain background	Reporter construct	Mutation/ Complement	Notes
<i>S. sonnei</i>	53G (Formal <i>et al.</i> , 1966)	NA	WT	-
<i>S. sonnei</i>	53G	GFP from pFPV25.1 (Valdivia <i>et al.</i> , 2006)	WT	Carb <sup>R</sup>
<i>S. sonnei</i>	53G	mCherry from pFPV-mcherry (Drecktrah <i>et al.</i> , 2008)	WT	Carb <sup>R</sup>
<i>S. sonnei</i>	381 (Watson <i>et al.</i> , 2018)	GFP from pFPV25.1	WT	Clinical isolate H140860381 from C. Jenkins, Public Health England; Carb <sup>R</sup>
<i>S. sonnei</i>	53G	GFP from pFPV25.1	$\Delta tssB$ (This study)	T6SS mutant with <i>tssB</i> replaced with <i>aphA-3</i> , conferring Kanamycin resistance; Carb <sup>R</sup> , Kan <sup>R</sup>
<i>S. sonnei</i>	53G	GFP from pFPV25.1	$\Delta g4c$ (Caboni <i>et al.</i> , 2015)	Capsule mutant with <i>g4c</i> cluster (genes <i>ymcDCBA</i> , <i>yccZ</i> , <i>etp</i> , <i>etk</i> ) replaced with <i>erm</i> cassette conferring resistance to Erythromycin; Carb <sup>R</sup> , Erm <sup>R</sup>
<i>S. sonnei</i>	53G	GFP from pFPV25.1	$\Delta mxid$ (Watson <i>et al.</i> , 2018)	T3SS mutant, with <i>mxid</i> replaced with <i>aphA-3</i> , conferring Kanamycin resistance; Carb <sup>R</sup> , Kan <sup>R</sup>
<i>S. sonnei</i>	53G	GFP from pFPV25.1	-pSS This study, based on (McVicker <i>et al.</i> , 2016)	Phase II <i>S. sonnei</i> , spontaneously cured from pSS virulence plasmid; Carb <sup>R</sup>
<i>S. sonnei</i>	53G	NA	$\Delta O-Ag$ (Watson <i>et al.</i> , 2019)	O-Antigen mutant, with O-Ag biosynthesis operon ( <i>wbg wzy</i> , IS630, <i>wbgVWXYZ</i> ) replaced with <i>aphA-3</i> , conferring Kanamycin resistance; Kan <sup>R</sup>
<i>S. sonnei</i>	53G	GFP from pFPV25.1	$\Delta O-Ag$	O-Antigen mutant with O-Ag biosynthesis operon ( <i>wbgTU</i> , <i>wzx</i> , <i>wzy</i> , IS630, <i>wbgVWXYZ</i> ) replaced with <i>aphA-3</i> , conferring kanamycin resistance; Carb <sup>R</sup> , Kan <sup>R</sup>
<i>S. sonnei</i>	53G	NA	$\Delta O-Ag^{+pSSO-Ag}$ (Watson <i>et al.</i> , 2019)	O-Ag biosynthesis operon ( <i>wzz</i> , <i>wbgTU</i> , <i>wzx</i> , <i>wzy</i> , IS630, <i>wbgVWXYZ</i> with 102 bp 5' of <i>wzz</i> and 76 bp 3' of <i>wbgZ</i> ) from 53G inserted into pSEVA471:SmR expressed in a <i>S. sonnei</i> 53G $\Delta O-Ag$ background; Kan <sup>R</sup> , Sm <sup>R</sup>
<i>S. flexneri</i>	M90T-sm (Sansone <i>et al.</i> , 1982)	GFP (Mostowy <i>et al.</i> , 2010)	WT	Serotype 5a; Carb <sup>R</sup> , naturally Sm <sup>R</sup>
<i>S. flexneri</i>	2457T (Formal <i>et al.</i> , 1958)	GFP from pFPV25.1	WT	Serotype 2a; Carb <sup>R</sup>

## References

- Formal SB, Kent TH, May HC, Palmer A, Falkow S, LaBrec EH. Protection of monkeys against experimental shigellosis with a living attenuated oral polyvalent dysentery vaccine. *J Bacteriol.* 1966 Jul;92(1):17-22.
- Valdivia RH, Falkow S. Bacterial genetics by flow cytometry: rapid isolation of *Salmonella typhimurium* acid-inducible promoters by differential fluorescence induction. *Mol Microbiol.* 1996 Oct;22(2):367-78.
- Drecktrah D, Levine-Wilkinson S, Dam T, Winfree S, Knodler LA, Schroer TA, Steele-Mortimer O. Dynamic behavior of *Salmonella*-induced membrane tubules in epithelial cells. *Traffic.* 2008 Dec;9(12):2117-29. doi:10.1111/j.1600-0854.2008.00830.x.
- Caboni M, Pédrón T, Rossi O, Goulding D, Pickard D, Citiulo F, MacLennan CA, Dougan G, Thomson NR, Saul A, Sansone PJ, Gerke C. An O antigen capsule modulates bacterial pathogenesis in *Shigella sonnei*. *PLoS Pathog.* 2015 Mar 20;11(3):e1004749. doi: 10.1371/journal.ppat.1004749.
- Watson J, Jenkins C, Clements A. *Shigella sonnei* does not use amoebae as protective hosts. *Appl Environ Microbiol.* 2018 Apr 16;84(9). pii: e02679-17. doi:10.1128/AEM.02679-17.
- McVicker G, Tang CM. Deletion of toxin-antitoxin systems in the evolution of *Shigella sonnei* as a host-adapted pathogen. *Nat Microbiol.* 2016 Nov 7;2:16204. doi:10.1038/nmicrobiol.2016.204.
- Watson JL, Sanchez-Garrido J, Goddard PJ, Torraca V, Mostowy S, Shenoy AR, Clements A. *Shigella sonnei* O-antigen inhibits internalisation, vacuole escape and inflammasome activation. *bioRxiv.* 2019, 799379; doi:10.1101/799379.
- Sansonetti PJ, Kopecko DJ, Formal SB. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect Immun.* 1982 Mar;35(3):852-60.

Mostowy S, Bonazzi M, Hamon MA, Tham TN, Mallet A, Lelek M, Gouin E, Demangel C, Brosch R, Zimmer C, Sartori A, Kinoshita M, Lecuit M, Cossart P. Entrapment of intracytosolic bacteria by septin cage-like structures. *Cell Host Microbe*. 2010 Nov 18;8(5):433-44. doi:10.1016/j.chom.2010.10.009.

Formal SB, Dammin GJ, Labrec EH, Schneider H. Experimental *Shigella* infections: characteristics of a fatal infection produced in guinea pigs. *J Bacteriol*. 1958 May;75(5):604-10.