Title: Prolonged outbreak of multidrug-resistant *Shigella sonnei* harbouring *bla*CTX-M-27 in Victoria, Australia

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Abstract

Objectives

In Australia, cases of shigellosis usually occur in returned travellers from shigellosis-endemic regions, or in men who have sex with men. Resistance to multiple antibiotics has significantly increased in *Shigella sonnei* and represents a significant public health concern. Here we investigate an outbreak of multidrug-resistant *S. sonnei* in Victoria, Australia.

Methods

We undertook whole genome sequencing of 54 extended-spectrum beta-lactamase (ESBL) producing *S. sonnei* received at the Microbiological Diagnostic Unit Public Health Laboratory between January 2019 and March 2020. The population structure and antimicrobial resistance profiles were identified by genomic analyses, with 73 previously characterised Australian *S. sonnei* to provide context. Epidemiological data including age and sex of the shigellosis cases were also collected.

Results

There was a significant increase in cases of ESBL *S. sonnei* from July 2019. Most of the ESBL *S. sonnei* (65%) fell within a single cluster, that was predominantly comprised of male cases, and were characterised by the presence of *bla*CTX-M-27 gene conferring resistance to extended-spectrum cephalosporins. These isolates were also multidrug-resistant, including resistance to azithromycin and co-trimoxazole and reduced susceptibility to ciprofloxacin.

Conclusions

Our data has uncovered a prolonged clonal outbreak of ESBL *S. sonnei* that was likely first introduced by returned travellers and has subsequently been circulating locally in Australia. The emergence of a local outbreak of ESBL *S. sonnei,* with a multidrug-resistant profile, including reduced susceptibility to ciprofloxacin, represents a significant public health threat.

52 **Introduction**

53 *Shigella* species are one of the leading causative agents for severe diarrhoeal disease
54 globally(1, 2). While the burden of disease is disproportionately experienced by children
55 under the age of five in low- and middle-income countries (LMICs)(1), in high-income
56 countries (HICs) cases of shigellosis are usually associated with either returned travellers or
57 in men who have sex with men (MSM)(3-5). Endemic shigellosis in men in HICs is often
58 considered a sexually transmitted infection (STI), with several *Shigella sonnei* and *Shigella*
59 *flexneri* lineages associated with MSM outbreaks(6-8).
60
61 A common characteristic of the MSM-associated outbreaks of *Shigella* infections is the
62 prevalence of multidrug resistance (MDR) to critical oral therapeutics; ciprofloxacin is the
63 first-line agent, with azithromycin or co-trimoxazole being second-line agents. Antimicrobial
64 resistance (AMR) to azithromycin and co-trimoxazole is usually mediated by the acquisition
65 of an MDR plasmid(7), while resistance to ciprofloxacin, reported in MSM-associated *S.*
66 *sonnei,* is due to point mutations in quinolone resistance determining regions (QRDRs)(5). In
67 the presence of resistance to oral agents, the most frequently used treatment option for
68 severe shigellosis is third-generation (extended-spectrum) cephalosporins such as
69 ceftriaxone or cefotaxime, which are given intravenously(9). Sporadic cases of extended-
70 spectrum beta-lactamase (ESBL) producing *S. sonnei* have been previously reported, often
71 in association with travel to Asia(6, 10, 11), but these have not been associated with
72 prolonged outbreaks.
73
74 Here, we investigated the recent increase of ESBL-resistant *S. sonnei* reported from late
75 2019 to early 2020 in the state of Victoria, Australia. We used whole-genome sequence
76 (WGS) data of *S. sonnei,* combining it with epidemiological data, and contextualising these
77 ESBL isolates with previously characterised Australian *S. sonnei* isolates, to demonstrate
78 the emergence of an ESBL-resistant lineage of *S. sonnei* circulating in males since October
79 2019.

Methods

Shigellosis is a notifiable disease in Australia. The Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) is the bacteriology reference laboratory for the State of Victoria (population approximately 6.4 million). MDU PHL receives *Shigella* isolates from primary pathology laboratories for the purpose of further characterisation, including phenotypic susceptibility testing and routine WGS. All *S. sonnei* received by the MDU PHL from 1 January 2019 to 31 March 2020 were assessed for the ESBL markers (resistance to ceftriaxone and presence of ESBL gene on WGS). The 54 ESBL-producing isolates identified also had associated epidemiological data including time of collection, sex and age of the patient. To compare ESBL *S. sonnei* notifications to a previous baseline period, seven sporadic ESBL *S. sonnei* received from 1 January 2019 to 30 May 2019 (previously published) were included(5). Details of the ESBL isolates are in **Supplementary Table 1,** and short read data are available at BioProject PRJNA319594.

DNA extracts from 47 novel ESBL isolates were prepared using Illumina Nextera XT DNA library chemistry and whole-genome sequenced on a NextSeq500 or NextSeq550. Sequences from 73 Australian *S. sonnei* broadly representative of the diversity of the previously-established population structure were included to provide a contextual framework for the ESBLs *S. sonnei*(4, 5). The 127 genomes were mapped to the reference *S. sonnei* (accession CP000038) to call single nucleotide polymorphisms (SNPs) using Snippy v.4.6.0, with filtering of phage regions identified using PHASTER(12), resulting in a core SNP alignment of 4,849 bases. A maximum likelihood (ML) phylogeny was inferred using IQTree (v.1.6.12)(13) and a GTR+G4 model. The resulting ML phylogeny was mid-point rooted with ape (v.5.3)(14) and phangorn (v.2.5.5)(15), before being visualised with ggtree (v.1.16.6)(16).

De novo assembly was performed using SPAdes (v.3.14.0)(17) using the '–isolate' flag. *In silico* determination of known AMR genes in the AMRfinderPlus database using abriTAMR (v.2020-01-22.1) (https://github.com/MDU-PHL/abritamr). Known point mutations in the QRDRs of *gyrA* and *parC* were identified from Snippy output. Pairwise SNP distances between isolates were determined using harrietR (v.0.2.3) (https://github.com/andersgs/harrietr) in R (v.3.6.1).

Results and Discussion

In total, 54 *S. sonnei* ESBL isolates were identified in Victoria in the 15 months between January 2019 and March 2020. The inferred population structure in **Figure 1A** shows the ESBL isolates were distributed within previously defined lineages(4). In the baseline period (January 2019 to May 2019), six isolates fell in Lineage 1 and one in Lineage 4. Of the 47 novel ESBL isolates received in the study period (June 2019 and March 2020), 35/47 (74.5%) fell in Lineage 3, while Lineage 1 and Lineage 4 each comprised six novel ESBL isolates (**Figure 1A**). The 35 ESBL Lineage 3 isolates formed a genomic cluster, highly suggestive of an outbreak, with a median pairwise distance of 3 SNPs (interquartile range 2-4 SNPs). These 35 putative outbreak isolates and two contextual isolates were characterised by the presence of the ESBL resistance gene *bla*CTX-M-27, accompanied by additional AMR determinants including *mph(A)* (azithromycin resistance), *dfrA1* and *sul2* (co-trimoxazole resistance), and decreased susceptibility to ciprofloxacin with a single point mutation in *gyrA* (S83L). Together these genes confer resistance to the critical oral antibiotics plus extended-spectrum cephalosporins, such as ceftriaxone.

There was a marked increase in ESBL *S. sonnei* in late 2019 and early 2020 compared to early 2019 with 43/54 (76%) of cases occurring from October 2019 onwards, (**Figure 1B**). The increase was predominately due to isolates carrying *bla*CTX-M-27, with both the number and proportion of such isolates increasing over the quarters (Q1-2019, 0/1 (0%); Q2-2019, 1/6 (17%); Q3-2019, 1/5 (20%); Q4-2019 13/19 (70%); Q1-2020, 23/23 (100%). All

but 3/38 *bla*CTX-M-27 were part of Lineage 3. The remaining three isolates with *bla*CTX-M-27 fell in Lineage 1 and were also characterised by three-point mutations in QRDRs. However, the diversity of the AMR profile and demographic characteristics combined with the relatively low incidence of ESBL cases in Lineage 1, suggest these ESBL isolates are likely to be sporadic introductions from different sources. Indeed, the ESBL isolates in Lineage 1 and Lineage 4 had greater diversity of *bla*CTX-M genes compared to Lineage 3, with the *bla*CTX-M-14 or *bla*CTX-M-15 the more common ESBL mechanisms (**Figure 1A-B**).

The population demographics of the cluster of *bla*CTX-M-27 genomes in Lineage 3 is notably different from the sporadic ESBL cases in other lineages and highly indicative of a prolonged outbreak event in Australia. Lineage 3 has been previously associated with a high proportion of cases where the identified primary risk factor was MSM(4), and in this study, 33/35 (94%) of cases were men (**Figure 1C**). The first case in the cluster occurred in September 2019, followed by 2-12 cases per month through to the end of the study period. The epidemic curve is highly suggestive of an outbreak event. Further, we note the AMR profile of these Australian ESBL isolates is consistent with that of a cluster of MDR *S. sonnei*, with the same ESBL gene *bla*CTX-M-27, that was detected in the United Kingdom between March and November 2018, and identified in a public health alert by Public Health England (PHE)(18). The PHE alert notes some of the ESBL *S. sonnei* isolates also clustered with isolates from cases in the USA from male patients who identified as MSM(18). While investigation of the global prevalence of ESBL *S. sonnei* was beyond the scope of this study, it does suggest the potential global dissemination of this ESBL sub-lineage and highlights the need for future public health surveillance to be able rapidly identify and classify high risk outbreak lineages. Notably, two contextual isolates, which had been previously characterised from returned travellers to south-east Asia(4), had the same AMR profile as the ESBL outbreak cluster. These two isolates were taken from female patients in 2017, which indicates this sub-lineage was circulating in south-east Asia at this time. This is

suggestive that this sub-lineage of ESBL *S. sonnei* may have been introduced to Australia by a returned traveller from this region, and then gone on to be locally transmitted.

Here we report the emergence of a prolonged outbreak of ESBL resistant *S. sonnei* in Victoria. This represents a significant public health threat with members of this prolonged outbreak now resistant to ceftriaxone, co-trimoxazole and azithromycin and reduced susceptibility to ciprofloxacin. The latent spread of this ESBL lineage in Victoria has likely occurred in populations with high antimicrobial exposure, coupled with high resistance potential with an existing QRDR mutation, and poses a significant concern for this lineage to become resistant to ciprofloxacin. This could have serious clinical implications, necessitating the use of extremely broad-spectrum antimicrobials such as carbapenems, and reducing the likelihood of a patient receiving the correct empiric therapy prior to the identification of the MDR *Shigella*. Our data also demonstrates the power of enhanced surveillance of enteric pathogens through genomic epidemiology and highlights the need for systematic reporting on ESBL resistance in *Shigella* species, which is not currently required in Australian public health laboratories.

Figure Legend

Figure 1: Population structure and antimicrobial resistance profiles of ESBL *Shigella sonnei*

A. The mid-point rooted phylogenetic tree of 54 ESBL *Shigella sonnei* and 73 contextual isolates. The tips are coloured by ESBL status for the novel isolates and by membership to previously established lineages for the contextual isolates. The sex of the patient is shown to the right of the phylogeny. Known genetic determinants for critical antimicrobials are shown as a heatmap. The * next to the gene indicates a partial match (partial gene recovery occurs when between 50% and 90% of a protein in the AMRfinder database is covered by a contig at >90% identity). B. Epidemic curve of ESBL *S. sonnei,* coloured by ESBL gene, received at MDU PHL between 1 January 2019 and 30 March 2020. C. The patient characteristics of the 35 *S. sonnei* isolates in ESBL outbreak lineage with the histogram stratified by age group and sex.

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Transparency declarations

None to declare.

References

1. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet 382:209–222.

2. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SR, Albertson SB, Stanaway JD, Deshpande A, Abebe Z, Alvis-Guzman N, Amare AT, Asgedom SW, Anteneh ZA, Antonio CAT, Aremu O, Asfaw ET, Atey TM, Atique S, Avokpaho EFGA, Awasthi A, Ayele HT, Barac A, Barreto ML, Bassat Q, Belay SA, Bensenor IM, Bhutta ZA, Bijani A, Bizuneh H, eda-Orjuela CAC, Dadi AF, Dandona L, Dandona R, Do HP, Dubey M, Dubljanin E, Edessa D, Endries AY, Eshrati B, Farag T, Feyissa GT, Foreman KJ, Forouzanfar MH, Fullman N, Gething PW, Gishu MD, Godwin WW, Gugnani HC, Gupta R, Hailu GB, Hassen HY, Hibstu DT, Ilesanmi OS, Jonas JB, Kahsay A, Kang G, Kasaeian A, Khader YS, Khalil IA, Khan EA, Khan MA, Khang Y-H, Kissoon N, Kochhar S, Kotloff KL, Koyanagi A, Kumar GA, Razek El HMA, Malekzadeh R, Malta DC, Mehata S, Mendoza W, Mengistu DT, Menota BG, Mezgebe HB, Mlashu FW, Murthy S, Naik GA, Nguyen CT, Nguyen TH, Ningrum DNA, Ogbo FA, Olagunju AT, Paudel D, Platts-Mills JA, Qorbani M, Rafay A, Rai RK, Rana SM, Ranabhat CL, Rasella D, Ray SE, Reis C, Renzaho AM, Rezai MS, Ruhago GM, Safiri S, Salomon JA, Sanabria JR, Sartorius B, Sawhney M, Sepanlou SG, Shigematsu M, Sisay M, Somayaji R, Sreeramareddy CT, Sykes BL, Taffere GR, Topor-Madry R, Tran BX, Tuem KB, Ukwaja KN, Vollset SE, Walson JL, Weaver MR, Weldegwergs KG, Werdecker A, Workicho A, Yenesew M, Yirsaw BD, Yonemoto N, Sayed Zaki El M, Vos T, Lim SS, Naghavi M, Murray CJ, Mokdad AH, Hay SI, Reiner RC Jr, Collaborators G2DD. 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 18:1211–1228.

3. Baker KS, Dallman TJ, Field N, Childs T, Mitchell H, Day M, Weill F-X, Lefevre S, Tourdjman M, Hughes G, Jenkins C, Thomson N. 2018. Genomic epidemiology of *Shigella* in the United Kingdom shows transmission of pathogen sublineages and determinants of antimicrobial resistance. Sci Rep 8: 7389

4. Ingle DJ, Easton M, Valcanis M, Seemann T, Kwong JC, Stephens N, Carter GP, Gonçalves da Silva A, Adamopoulos J, Baines SL, Holt KE, Chow EPF, Fairley CK, Chen MY, Kirk MD, Howden BP, Williamson DA. 2019. Co-circulation of Multidrug-resistant *Shigella* Among Men Who Have Sex With Men in Australia. Clin Infect Dis 69: 1535-1544.

5. Williamson DA, Ingle DJ, Howden BP. 2019. Extensively Drug-Resistant Shigellosis in Australia among Men Who Have Sex with Men. N Engl J Med 381: 2477–2479.

6. Mook P, McCormick J, Bains M, Cowley LA, Chattaway MA, Jenkins C, Mikhail A, Hughes G, Elson R, Day M, Manuel R, Dave J, Field N, Godbole G, Dallman T, Crook P. 2016. ESBL-Producing and Macrolide-Resistant *Shigella sonnei*

Infections among Men Who Have Sex with Men, England, 2015. Emerg Infect Dis 22:1948–1952.

7. Baker KS, Dallman TJ, Field N, Childs T, Mitchell H, Day M, Weill F-X, Lefèvre S, Tourdjman M, Hughes G, Jenkins C, Thomson N. 2018. Horizontal antimicrobial resistance transfer drives epidemics of multiple *Shigella* species. Nat Commun 9: 1462.

8. Bardsley M, Jenkins C, Mitchell HD, Mikhail AFW, Baker KS, Foster K, Hughes G, Dallman TJ. 2020. Persistent Transmission of Shigellosis in England Is Associated with a Recently Emerged Multidrug-Resistant Strain of *Shigella sonnei*. J Clin Microbiol 58:909–11.

9. Antibiotic Expert Group. 2019. Acute infectious diarrhoea. *In* eTG complete digital. Melbourne. Available at: https://www.tg.org.au.

10. Hrabak J, Empel J, Gniadkowski M, Halbhuber Z, Rebl K, Urbaskova P. 2008. CTX-M-15-Producing *Shigella sonnei* Strain from a Czech Patient Who Traveled in Asia. J Clin Microbiol 46:2147–2148.

11. Lee W, Chung H-S, Lee H, Yum JH, Yong D, Jeong SH, Lee K, Chong Y. 2013. CTX-M-55-Type Extended-Spectrum β-lactamase-Producing *Shigella sonnei* Isolated from a Korean Patient Who Had Travelled to China. Ann Lab Med 33:141–4.

12. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21.

13. Nguyen LT, Schmidt HA, Haeseler von A, Minh BQ. 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32:268–274.

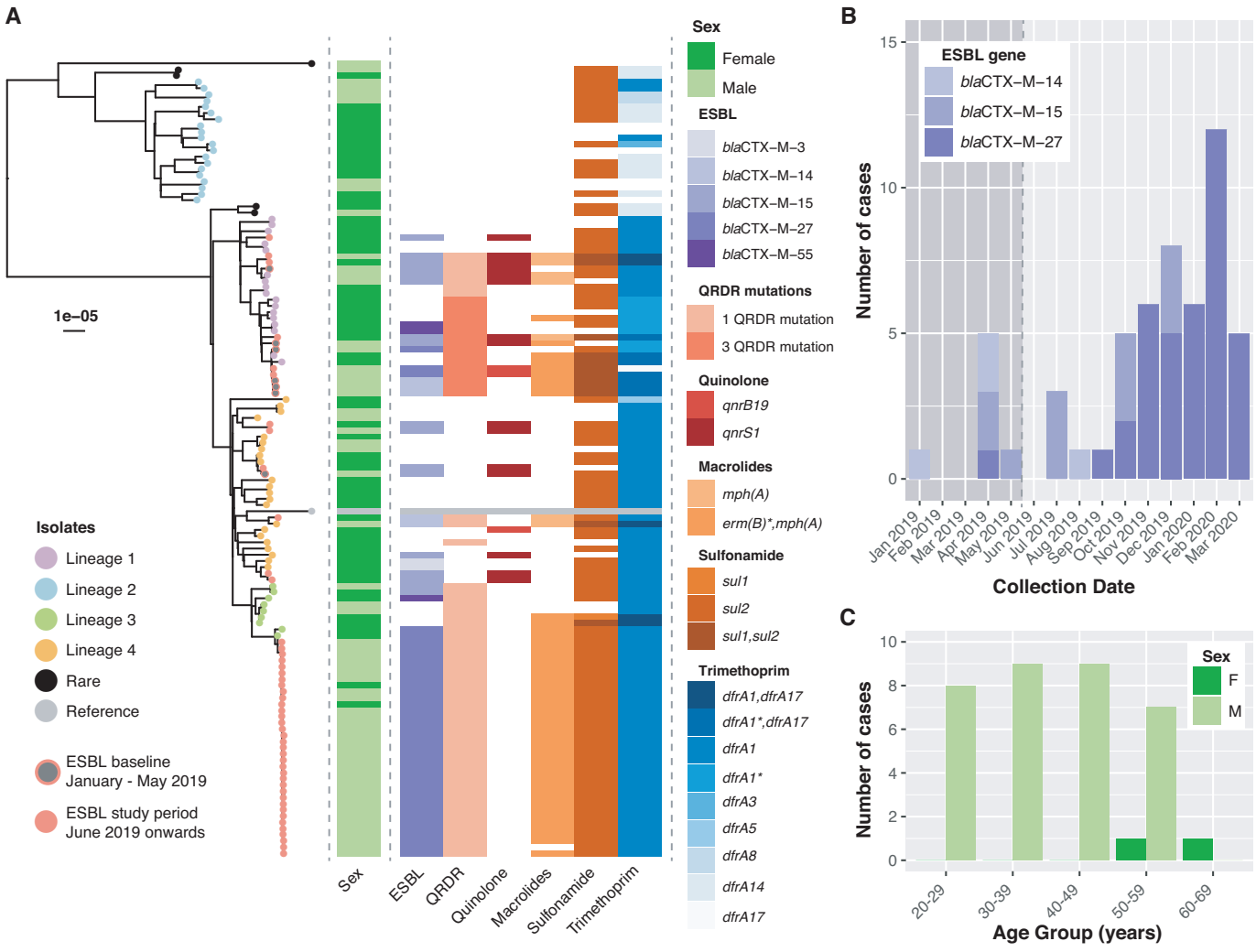
14. Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

15. Schliep KP. 2010. phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593.

16. Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y. 2016. ggtree: an rpackage for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol, 8:28–36.

17. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell Sequencing. J Comput Biol 19:455–477.

18. Public Health England. 2019. MDR *Shigella sonnei* cluster (*CTX-M-27*) probably associated with MSM. PHE Publications.



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