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Co-circulation of Multidrug-resistant *Shigella* Among Men Who Have Sex With Men in Australia

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Background. In urban Australia, the burden of shigellosis is either in returning travelers from shigellosis-endemic regions or in men who have sex with men (MSM). Here, we combine genomic data with comprehensive epidemiological data on sexual exposure and travel to describe the spread of multidrug-resistant *Shigella* lineages.

Methods. A population-level study of all cultured *Shigella* isolates in the state of Victoria, Australia, was undertaken from 1 January 2016 through 31 March 2018. Antimicrobial susceptibility testing, whole-genome sequencing, and bioinformatic analyses of 545 *Shigella* isolates were performed at the Microbiological Diagnostic Unit Public Health Laboratory. Risk factor data on travel and sexual exposure were collected through enhanced surveillance forms or by interviews.

Results. Rates of antimicrobial resistance were high, with 17.6% (95/541) and 50.6% (274/541) resistance to ciprofloxacin and azithromycin, respectively. There were strong associations between antimicrobial resistance, phylogeny, and epidemiology. Specifically, 2 major MSM-associated lineages were identified: a *Shigella sonnei* lineage (n = 159) and a *Shigella flexneri* 2a lineage (n = 105). Of concern, 147/159 (92.4%) of isolates within the *S. sonnei* MSM-associated lineage harbored mutations associated with reduced susceptibility to recommended oral antimicrobials: namely, azithromycin, trimethoprim-sulfamethoxazole, and ciprofloxacin. Long-read sequencing demonstrated global dissemination of multidrug-resistant plasmids across *Shigella* species and lineages, but predominantly associated with MSM isolates.

Conclusions. Our contemporary data highlight the ongoing public health threat posed by resistant *Shigella*, both in Australia and globally. Urgent multidisciplinary public health measures are required to interrupt transmission and prevent infection.

Keywords. shigellosis; epidemiology; genomics; antimicrobial resistance; sexually transmitted infections.

Shigellosis is estimated to cause 190 million cases of diarrhea globally per year [1]. In low- and middle-income countries, the burden of shigellosis is concentrated in children, with inadequate sanitation and contaminated food and/or water the most common modes of acquisition. In contrast, shigellosis in high-income countries occurs predominantly in returning travelers or in men who have sex with men (MSM) [2, 3]. In many high-income countries, there are increasing reports of locally acquired shigellosis in MSM, with endemic shigellosis in males in these countries often considered a sexually transmitted infection (STI) [2, 4–8].

As in other countries, treatment for shigellosis in Australia is recommended, to reduce both symptoms and asymptomatic bacterial shedding. The recommended first-line oral treatment is ciprofloxacin and, as second-line agents, azithromycin or trimethoprim-sulfamethoxazole (co-trimoxazole) are favored [1]. However, recent studies have highlighted increasing resistance to these agents amongst *Shigella* spp., in low-, middle-, and high-income countries [9–13]. Indeed, the World Health Organization and Centers for Disease Control and Prevention have declared antimicrobial-resistant (AMR) *Shigella* spp. to be a major public health threat [14, 15]. Of further concern is a recent advisory from the Centers for Disease Control and Prevention suggesting that ciprofloxacin may not be suitable for treatment in *Shigella* isolates with minimum inhibitory concentrations (MICs) of 0.12–1.0 μg/mL [16]. Although current interpretive criteria categorize isolates with MICs ≤1.0 μg/mL as susceptible [17], this advisory suggested isolates with MICs of 0.12–1.0 μg/mL may harbor ≥1 mutations associated with fluoroquinolone resistance, which could lead to adverse clinical outcomes and sustained shedding if fluoroquinolones are

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used for treatment [16]. Further, there are increasing reports of azithromycin resistance in *Shigella* spp. (mainly amongst MSM in urban populations), where azithromycin resistance is largely mediated by the plasmid-encoded *mph(A)* gene [9, 10, 13].

Whole-genome sequencing (WGS) has been previously used to describe both the global and regional molecular epidemiology of shigellosis. To date, studies have predominantly assessed either the population structure of individual *Shigella* lineages [18, 19] or have focused on representative subsamples of epidemiologically suspected epidemics [9, 10, 20]. Unbiased WGS of all cultured *Shigella* isolates in a population could provide valuable insights into possible transmission networks of shigellosis, in addition to providing information on emerging genotypic AMR patterns. Accordingly, we performed WGS of all cultured *Shigella* isolates in the state of Victoria, Australia, over a 2-year period. We combined genomic data with comprehensive epidemiological data on sexual exposure and travel to demonstrate the spread of highly resistant *Shigella* lineages within MSM. Further, we contextualized our isolates with data from recent studies of shigellosis in MSM and travelers, and demonstrate the global dissemination of highly related, multidrug-resistant plasmids.

METHODS

Setting, Bacterial Isolates, and Microbiological Testing

In Australia, shigellosis is a notifiable disease under public health legislation, and diagnostic laboratories forward *Shigella* isolates to a reference laboratory. The Microbiological Diagnostic Unit Public Health Laboratory is the bacteriology reference laboratory for the state of Victoria, covering a resident population of approximately 6.24 million people. Here, we conducted a retrospective, observational study of all cases of shigellosis associated with a *Shigella* isolate in Victoria between 1 January 2016 and 31 March 2018. Susceptibility testing was performed as detailed in the Supplementary Methods. We classified isolates with a ciprofloxacin MIC of 0.12–1 μg/mL as high resistance potential (HRP) isolates [21].

Epidemiological Investigation

Where possible, all cases of shigellosis in Victoria are interviewed by a public health officer; otherwise, notifying medical practitioners complete an enhanced surveillance questionnaire. Exposure information is collected for the 2 weeks prior to the illness' onset. Primary risk factors are considered in 1 of 3 main categories: (1) male to male sexual contact within Australia; (2) international travel; or (3) other/unknown risk factor. Cases are classified as an unknown source only when no risk factors have been identified after the interview. Travel destinations are categorized using the Standard Australian Classification of Countries, 2nd edition [22]. Our information on shigellosis notifications in Victoria was obtained from the National Notifiable Diseases Surveillance System (http://www9.health.gov.au/cda/source/cda-index.cfm). Data were collected in accordance with the Victorian Public Health and Wellbeing Act 2008 [23], and formal ethical approval was not required, as this work was part of enhanced epidemiological surveillance.

Whole-genome Sequencing and Bioinformatic Analyses

DNA extraction and WGS of study isolates were performed at the Microbiological Diagnostic Unit Public Health Laboratory (Supplementary Methods). In brief, reads were aligned to a reference genome to identify single nucleotide polymorphisms (SNPs) using Snippy v4.3.5 (https://github.com/tseemann/snippy), with filtering of phage regions identified using PHASTER [24] and recombinant regions identified using Gubbins v2.3.4 [25]. The final core SNPs were extracted with SNP sites [26].

A maximum likelihood phylogeny was produced using IQ-tree (v1.6.5) [27] and the population structure was investigated using a hierarchical Bayesian Analysis of Population Structure (BAPS) [28]. For context, we included data from other WGS-based studies of *Shigella* [10, 19, 29, 30]. We specifically included these previous studies, as the epidemiological context (MSM and/or travel) was similar to our study. *De novo* assembly was performed using Unicycler (v.0.4.6) [31], and AMR genes were detected using ABRicate (https://github.com/tseemann/abricate). Point mutations in the quinolone resistance determining region (QRDR) were detected by read mapping (Supplementary Methods). We selected 4 genomes for PacBio long-read sequencing (details in Supplementary Methods). Sequencing data are available at the National Center for Biotechnology Information Short Read Archive (BioProject PRJNA319594) and long read assemblies available at ENA (BioProject PRJEB30677).

Statistical Analysis

Comparisons between isolates or clusters were made using a chi-squared test. The Mann-Whitney Rank sum test was used to compare non-normal distributions. All statistical analyses were performed using R (version 3.4.0).

RESULTS

Epidemiological Characteristics of Cases and Antimicrobial Susceptibility Patterns

In total, 545 *Shigella* isolates (364 *Shigella sonnei* and 181 *Shigella flexneri*) isolates underwent WGS, representing 42.9% (545/1269) of shigellosis notifications in Victoria over the study period. The median age was 35 years (interquartile range 27–48; range 0–83), with a male to female ratio of 2.4:1.0 (Supplementary Figure 1). Over one-third of cases (36.0%; 196/545) were associated with overseas travel as a primary risk factor, and 176 (32.3%) cases identified MSM as a primary risk factor. There were 541 isolates that underwent susceptibility testing (Supplementary Table 1). Decreased susceptibility to azithromycin was identified in 51.4% (93/181) of *S. flexneri* and

50.3% (181/360) of *S. sonnei* ($P = .81$) cases, and resistance to ciprofloxacin in 9.4% (17/181) of *S. flexneri* and 21.7% (78/360) of *S. sonnei* ($P < .001$) cases. *S. sonnei* isolates were significantly more likely to be resistant to either 2 or 3 oral antimicrobials than *S. flexneri* (62.2% vs 40.9%, [$P < .001$] and 6.9% vs 0.6% [$P < .001$], respectively; Supplementary Table 1).

Correlation of Phylogeny With Epidemiological Risk Factors for Resistant Shigella spp.

Isolates were interrogated phylogenetically by species, and clustering was assessed using BAPS. Amongst *S. sonnei*, 4 major BAPS groups were identified (BP1–BP4; Table 1; Figure 1; Supplementary Figures 2–3). An additional cluster (BP5) represented grouping of the most divergent isolates, in a known limitation of this clustering approach [28]. As such, we did not consider this a biologically relevant grouping, and this group was excluded from further analyses.

Correlating the phylogeny with epidemiological characteristics revealed clear links with risk factors. Specifically, cases in BP1, BP2, and BP4 were associated with overseas travel (Table 1; Supplementary Figure 2), with strong associations between sub-lineages and regions of acquisition. For example, of the 32 cases of *S. sonnei* reporting travel to Southern or Central Asia, 32 cases (100%) were associated with BP1 (Supplementary Figure 4). In keeping with this observation, isolates from BP1 clustered with representative isolates from the Asian ciprofloxacin-resistant *S. sonnei* lineage III, previously described by Chung et al [19]. Further, in the BP1 lineage, isolates were significantly associated with ciprofloxacin resistance (Figure 1; Table 1); of the 75 ciprofloxacin-resistant isolates in BP1, 74 isolates harbored triple mutations in the QRDR (Figure 1).

In contrast to the travel-associated lineages, *S. sonnei* cases in BP3 were significantly associated with MSM, with 61% (97/159) of males in this lineage reporting MSM as a primary risk factor (Figure 1; Table 1). Isolates in BP3 were highly related, with a median pairwise core SNP distance of 4 (interquartile range 2–6; Table 1; Supplementary Figure 3). Further, MSM cases in BP3 were distributed across the study period and not limited to a temporally restricted outbreak (Supplementary Figure 2). Most isolates in BP3 (139/159; 87%) harbored highly related plasmids (see below) containing AMR determinants for azithromycin, trimethoprim, and sulfonamides (Figure 1). Although isolates in this lineage were not associated with ciprofloxacin resistance at the current Clinical and Laboratory Standards Institute breakpoint of 1 μg/mL, reducing the breakpoint to 0.12 μg/mL resulted in 147/149 (99%) of tested isolates being re-classified as having HRP for ciprofloxacin. All 149 isolates harbored single mutations in the QRDR (147 *gyrA* S83L and 2 *gyrA* D87Y; Figure 1).

Amongst *S. flexneri,* there were 4 major BAPS groups (BP1–BP4; Table 2; Supplementary Figure 3). These groups broadly correlated with serotyping, with the largest group (BP2) corresponding to *S. flexneri* serotype 2a (Supplementary Dataset).

Table 1. Characteristics of *Shigella sonnei* Isolates Included in This Study and Associations With Phylogenetic Groupings

Characteristic	BAPS Group[a]				*P*[b]
	BP1	BP2	BP3	BP4	
Median pairwise SNP difference (IQR)	50 (41–75)	168 (144–189)	4 (2–6)	106 (64–120)	
Phenotypic resistance	Number resistant / isolates tested (% resistant)				
Ampicillin	37/98 (37.8)	10/40 (25)	150/159 (94.3)	11/57 (19.3)	<.001
Ciprofloxacin	75/98 (76.5)	0/40	2/159 (1.3)	1/57 (1.8)	<.001
Azithromycin	27/98 (27.6)	0/40	148/159 (93.1)	6/57 (10.5)	<.001
Ceftriaxone	19/98 (19.4)	0/40	11/159 (6.9)	5/57 (8.8)	.002
Trimethoprim	97/98 (99.0)	26/40 (65.0)	159/159 (100)	57/57 (100)	<.001
Sulfathiozole	86/98 (87.8)	27/40 (67.5)	157/159 (98.7)	44/57 (77.2)	<.001
Gentamicin	3/97 (3.1)	0/40 (0)	0/159 (0)	1/57 (1.8)	.13
Meropenem	0/98 (0)	0/40 (0)	0/159 (0)	0/57	NA
Resistant to 2 oral antimicrobials	71/98 (72.4)	0/40 (0)	147/159 (92.4)	6/57 (10.5)	<.001
Resistant to 3 oral antimicrobials	22/98 (22.4)	0/40 (0)	2/159 (1.3)	1/57 (1.8)	<.001
High resistance potential for ciprofloxacin	82/88 (93.2)	0/39 (0)	147/149 (98.6)	12/52 (23.1)	<.001
Epidemiological characteristic					
Males (% BAPS group)	54/102 (52.0)	17/40 (42.5)	144/159 (90.6)	25/57 (43.8)	<.001
Median age (IQR)	28.5 (10.5–43)	36.5 (25.25–44.75)	38 (30–48)	34 (24–55)	<.001
Overseas travel or contact with traveler (% BAPS group)	62/102 (60.7)	25/40 (62.5)	12/159 (7.6)	39/57 (68.4)	<.001
Male-to-male sexual contact (% BAPS group)	8/102 (7.8)	3/40 (7.6)	97/159 (61.0)	2/57 (3.5)	<.001
Other/unknown (% BAPS group)	32/102 (31.3)	12/40 (30.0)	50/159 (31.4)	16/57 (28.1)	.49

Abbreviations: BAPS, Bayesian Analysis of Population Structure; BP, BAPS group; IQR, interquartile range; NA, not applicable; SNP, single-nucleotide polymorphism.

[a]There were 4 isolates that belonged to a divergent group (BP5) that were excluded from this analysis (see text). Also, 2 Australian isolates initially identified as BP4 were excluded from the BP4 analysis.

[b]As determined through a 2 x 4 χ^2 test.

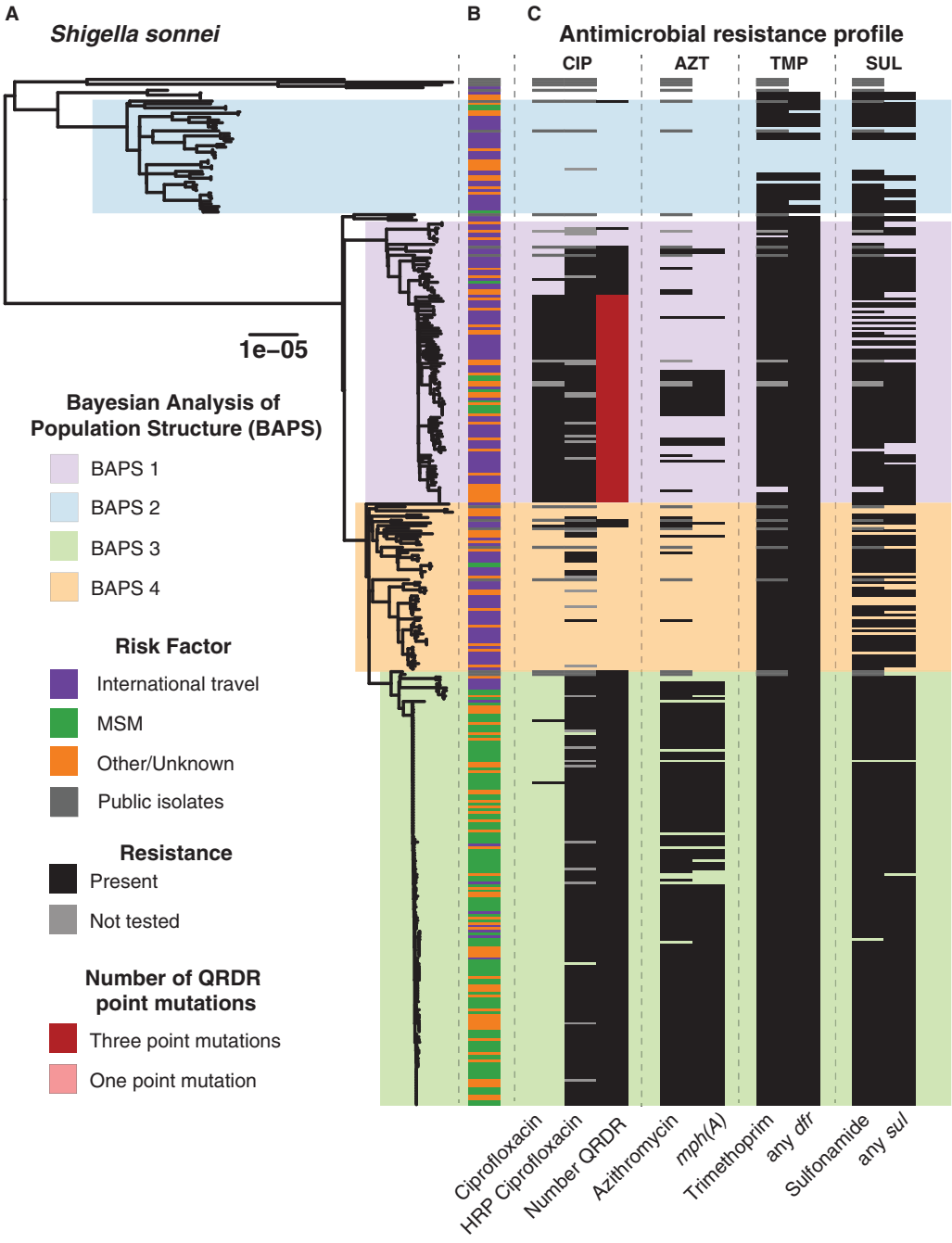

Figure 1. Population structure of 364 Australian *Shigella sonnei* isolates included in this study. *A*, The midpoint rooted phylogenetic tree of *Shigella sonnei* is plotted on the left. A total of 16 additional representative genomes are included as references to the established population structure [29]. BAPS groups are highlighted. *B*, Primary risk factors are shown. *C*, Phenotypic resistance profiles are shown next to their corresponding genotypic mutations for ciprofloxacin (number of QRDR mutations; see text), azithromycin (*mphA*), trimethoprim (any *dfr* gene), and sulfonamides (any *sul* gene). In addition, the effect of reducing the ciprofloxacin breakpoint to 0.12 µg/mL is displayed (HRP). Abbreviations: AZT, azithromycin; BAPS, Bayesian Analysis of Population Structure; CIP, ciprofloxacin; HRP, high resistance potential; MSM, men who have sex with men; QRDR, quinolone resistance determining region; SUL, sulfamethoxazole; TMP, trimethoprim.

An additional cluster (BP5), a grouping of divergent isolates, was excluded from further analyses. Similar to *S. sonnei,* there were distinct associations between phylogeny, AMR, and risk factors. Specifically, MSM status was associated with BP2 (58.4% [52/89] of males in this lineage were MSM) and, to a lesser extent, BP1 and BP3 (Table 2), whilst BP4 was more associated with recent travel (Table 2; Figure 2). In contrast to the major BP3 MSM lineage in *S. sonnei*, the BP2 MSM lineage in *S. flexneri* was not associated with ciprofloxacin resistance at a lower breakpoint of 0.12 µg/mL and did not harbor QRDR mutations (Figure 2; Supplementary Dataset). However, 63.8% (67/105) of isolates in the BP2 MSM lineage of *S. flexneri*

Table 2. Characteristics of *Shigella flexneri* Isolates Included in This Study and Associations With Phylogenetic Groupings

Characteristic	BAPS Group[a]				*P*[b]
	BP1	BP2	BP3	BP4	
Median pairwise SNP difference (IQR)	162 (125–181)	139 (6–283)	91 (70.25–615)	259 (118.25–325.75)	
Phenotypic resistance	Number resistant / isolates tested (% resistant)				
Ampicillin	26/32 (81.3)	102/105 (97.1)	15/16 (93.8)	6/10 (60.0)	<.001
Ciprofloxacin	2/32 (6.3)	12/105 (11.4)	0/16 (23.5)	2/10 (20.0)	.30
Azithromycin	12/32 (37.5)	74/105 (70.5)	6/16 (37.5)	0/10 (0)	<.001
Ceftriaxone	0/32 (0)	1/105 (1.0)	0/16 (0)	0/10 (0)	.86
Trimethoprim	24/32 (75.0)	92/105 (87.6)	2/16 (12.5)	7/10 (70.0)	<.001
Sulfathiozole	20/32 (62.5)	67/105 (63.8)	3/16 (18.6)	6/10 (60.0)	.008
Gentamicin	1/32 (3.1)	0/105 (0)	0/16 (0)	0/10 (0)	.25
Meropenem	0/32 (0)	0/105 (0)	0/16 (0)	0/10 (0)	NA
Resistant to 2 oral antimicrobials	13/32 (40.6)	58/105 (55.2)	0/16 (0)	2/10 (20.0)	<.001
Resistant to 3 oral antimicrobials	0/32 (0)	1/105 (1.0)	0/16 (0)	0/10 (0)	.91
High resistance potential for ciprofloxacin	18/29 (62.1)	14/97 (14.4)	0/15 (0)	6/10 (60.0)	<.001
Epidemiological characteristic					
Males (% BAPS group)	25/32 (78.1)	89/105 (84.8)	13/16 (81.3)	5/10 (50.0)	.001
Median age (IQR)	39 (30.25–47.75)	37 (28–50)	35.5 (30.25–49.25)	30 (7.25–38)	.19
Overseas travel or contact with traveler (% BAPS group)	11/32 (34.4)	19/105 (18.0)	6/16 (37.5)	6/10 (60.0)	.008
Male-to-male sexual contact (% BAPS group)	7/32 (21.9)	52/105 (49.5)	6/16 (37.5)	1/10 (10.0)	.003
Other/unknown (% BAPS group)	14/32 (43.8)	34/105 (32.4)	4/16 (25.0)	3/10 (30.0)	.05

Abbreviations: BAPS, Bayesian Analysis of Population Structure; BP, BAPS group; IQR, interquartile range; NA, not applicable; SNP, single-nucleotide polymorphism.

[a]There were 8 isolates that belonged to divergent group (BP5) and were excluded from this analysis (see text). Also, 10 isolates that belonged to distant serotype 6 were excluded.

[b]As determined through a 2 x 4 χ^2 test.

harbored azithromycin-resistant plasmids, similar to those in the *S. sonnei* BP3 MSM lineage (see below; Figures 2 and 3).

For context, we undertook combined analyses of 691 *S. sonnei* isolates (364 Australian and 327 international) and 408 *S. flexneri* isolates (171 Australian and 237 international; Supplementary Figures 4 and 5). For both Australian and United Kingdom *S. sonnei* and *S. flexneri*, there was limited clustering of isolates according to the region of travel (Supplementary Figures 4 and 5). Australian *S. sonnei* MSM isolates in BP3 formed a distinct lineage, almost completely comprising Australian isolates (Supplementary Figure 4). In contrast, for *S. flexneri*, international serotype 2a isolates in the previously described MSM clades from Baker et al [10] clustered together in the phylogeny with Australian isolates in the MSM-associated BP2 lineage (Supplementary Figure 5). Moreover, 31 of these international *S. flexneri* isolates also harbored pKSR100 plasmids, similar to those described from the United Kingdom (see below; Figure 3), further suggesting intercontinental dissemination of the MSM-associated *S. flexneri* 2a lineage.

Global Dissemination of Multidrug-resistant Plasmids Across Shigella Species and Lineage

To understand the relatedness of multidrug-resistant plasmids in our study, specifically amongst MSM-associated isolates, long-read sequencing was performed on 4 genomes, representing *S. flexneri* BP1 and BP2 and *S. sonnei* BP1 and BP3 (Supplementary Dataset). All 4 plasmids harbored bla_{TEM-1}, *ermB*, and *mph(A)*, and 3 of the plasmids contained an integron with additional AMR genes: namely, *sul1, dfrA17*, and *aadA5* (Figure 3). Comparison with pKSR100 (a conjugative plasmid previously identified in MSM-associated *Shigella* in the United Kingdom [7, 10]) revealed a high level of homology between pKSR100 and all 4 plasmids (Figure 3). The global relationship of pKSR100 plasmids was further explored by comparing all pKSR100-like plasmids (from our study and other recent work [10, 13]) to pKSR100 (Figure 4). The pKSR100-like plasmids were disseminated across *Shigella* species, but were predominantly associated with MSM-associated shigellosis, both in Australia and overseas (Figure 4).

DISCUSSION

In this study, we demonstrated that the local and global dissemination of clinically significant AMR *Shigella* spp. is driven largely by the spread of highly related, multidrug-resistant plasmids that are not restricted by *Shigella* species or lineage. By sampling all isolates in a population (rather than representatively sub-sampling known outbreaks) and integrating them with epidemiological data, we identified distinct correlations between *Shigella* sub-lineages and the 2 major modes of shigellosis acquisition in high-income countries: namely, international travel and

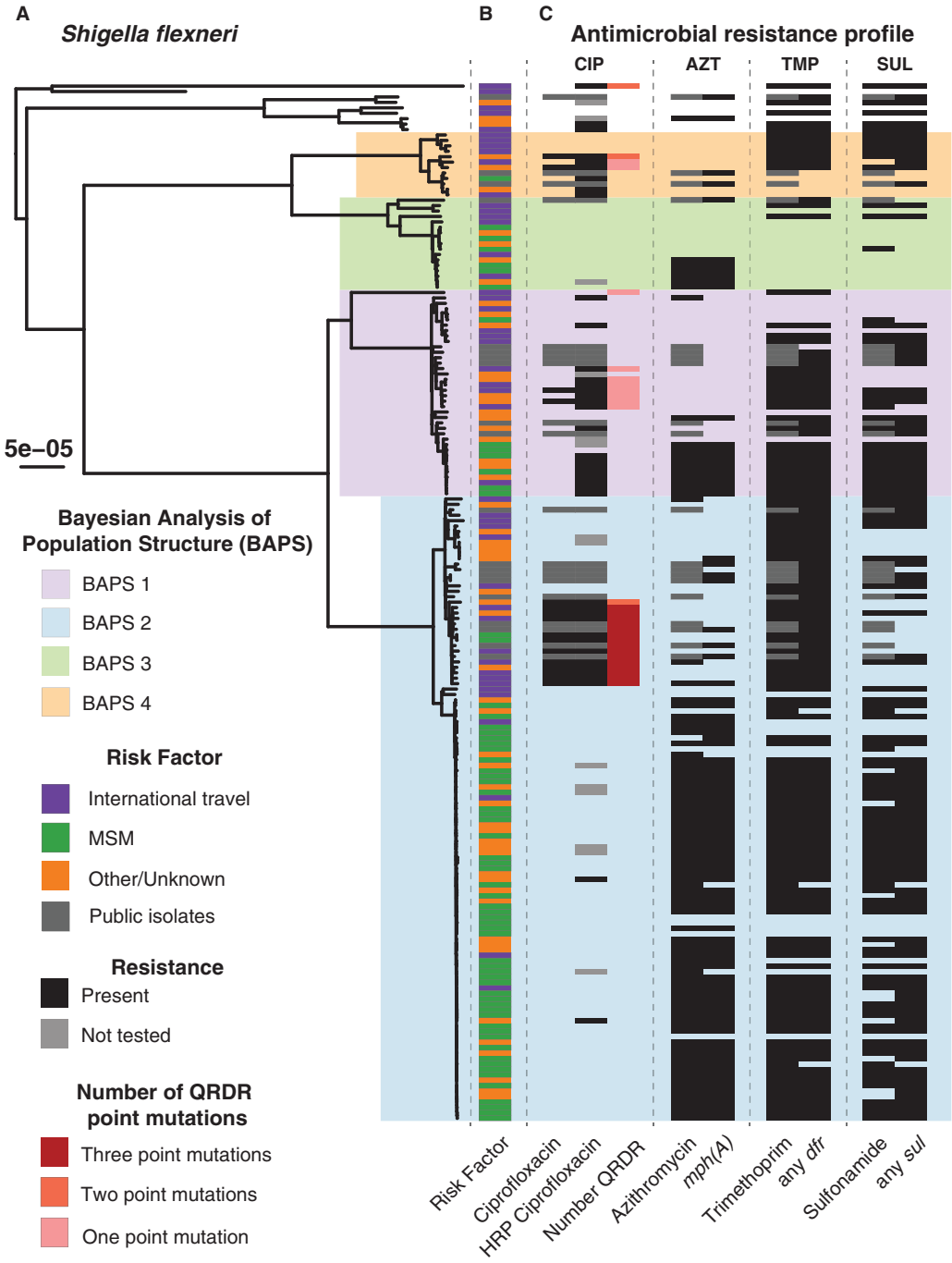

Figure 2. Population structure of 171 Australian *Shigella flexneri* isolates and 20 additional representative genomes are included as references to the established population structure [28]. *A*, The midpoint rooted phylogenetic tree of *Shigella flexneri* is plotted on the left. BAPS groups are highlighted. *B*, Primary risk factors are shown. *C*, Phenotypic resistance profiles are shown next to corresponding genotypic mutations for ciprofloxacin (number of QRDR mutations; see text), azithromycin (*mphA*), trimethoprim (any *dfr* gene), and sulfonamides (any *sul* gene). In addition, the effect of reducing the ciprofloxacin breakpoint to 0.12 µg/mL is displayed (HRP). Abbreviations: AZT, azithromycin; BAPS, Bayesian Analysis of Population Structure; CIP, ciprofloxacin; HRP, high resistance potential; MSM, men who have sex with men; QRDR, quinolone resistance determining region; SUL, sulfamethoxazole; TMP, trimethoprim.

domestically acquired MSM-associated shigellosis, each associated with approximately one-third of all cases in this study.

Of specific concern was the high prevalence of resistance to those oral agents currently recommended for the treatment of shigellosis. Approximately 50% of all isolates displayed resistance to azithromycin, representing, to date, the highest reported azithromycin resistance rate in *Shigella* spp. globally [13, 32, 33]. Notably, azithromycin resistance was significantly more common in MSM-associated *Shigella* (93% in BP3 *S. sonnei* and 71% in BP2 *S. flexneri*), signalling the demise of azithromycin as

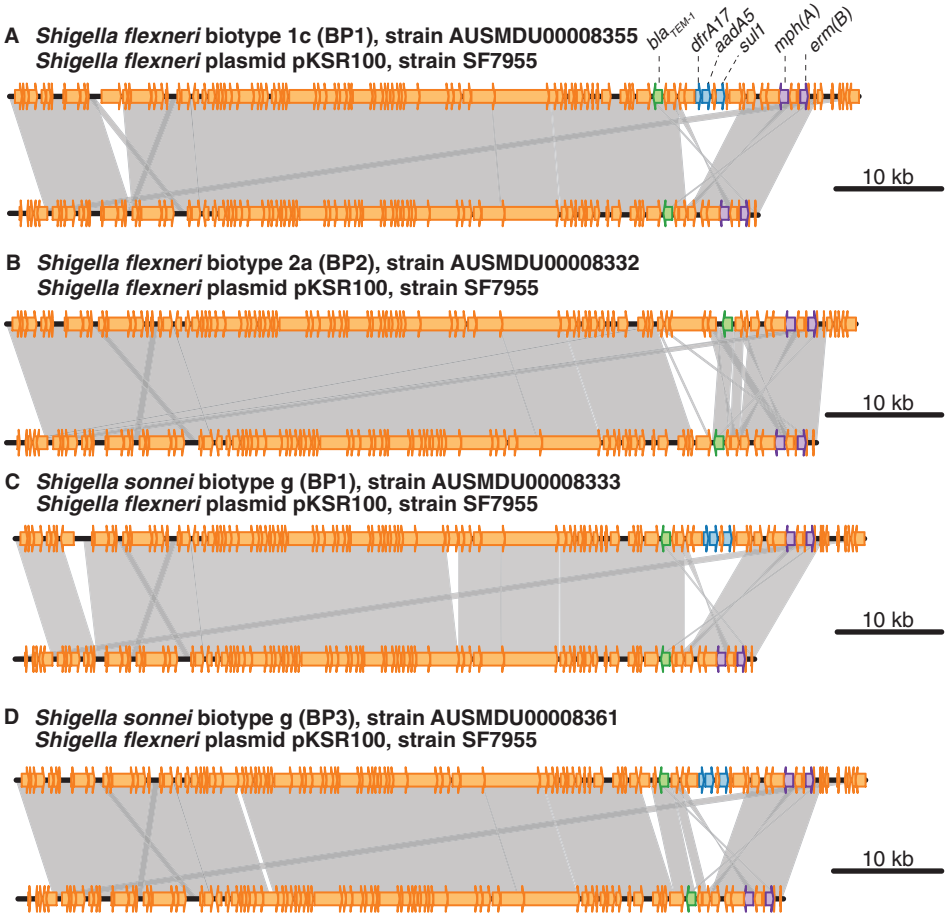

Figure 3. Comparison of 4 Australian pKSR100-like plasmids with pKSR100 (SF7955). The structure of the pKSR100 plasmid is shown at the bottom of each panel, with the comparison plasmid at the top. *A, Shigella flexneri* biotype 1c strain AUSMDU00008355 (BP1 lineage); *B, S. flexneri* biotype 2a strain AUSMDU00008332 (BP2 lineage); *C, Shigella sonnei* biotype g strain AUSMDU00008333 (BP1 lineage); and *D, S. sonnei* biotype g strain AUSMDU00008361 (BP3 lineage). Plasmid genes are shown in orange, with genes mediating resistance to different drug classes highlighted in green (bla_{TEM-1}), purple (*mphA* and *ermB*), and blue (*dfrA17, sul1*, and *aadA*). The shaded gray indicates regions with nucleotide homologies of ≥95%. Abbreviation: BP, Bayesian Analysis of Population Structure group.

a suitable treatment option for domestically acquired shigellosis in MSM in our population. The reasons for this high rate of azithromycin resistance are multifactorial and are likely related to both host and pathogen factors. First, the recommended use of azithromycin as an empiric agent for the syndromic treatment of urethritis is likely to have exerted selection pressure for the emergence of azithromycin resistance in MSM [34]; this hypothesis is corroborated by the contemporaneous emergence of azithromycin resistance in other sexually transmitted pathogens, such as *Neisseria gonorrhoeae*, *Treponema pallidum*, and *Mycoplasma genitalium* [35–38]. Second, the finding that *mph(A)* was harbored on genetically similar pKSR100-like plasmids, regardless of species or lineage, highlights the apparent ease of horizontal transmission of these particular plasmids; this observation is supported by the identification of similar plasmids from multiple geographic locations and epidemiological contexts [7, 9, 10, 13]. Collectively, our data support the existence of a global outbreak of a highly successful azithromycin resistance plasmid, predominantly in MSM populations.

Compared to azithromycin resistance, ciprofloxacin resistance was largely associated with international travel to areas of high endemicity (particularly Southeast and Central Asia), rather than local populations, in keeping with the fact that ciprofloxacin is a restricted antibiotic in Australia [39]. However, when an MIC threshold of 0.12 µg/mL was applied, approximately 57% of all tested isolates in our study were classified as HRP, compared to only 18% at the current resistance breakpoint of >1.0 µg/mL. The difference was largely driven by the MSM-associated *S. sonnei* BP3 lineage, in which all HRP isolates harbored single mutations (mainly *gyrA* S83L) in the QRDR. This finding is of specific concern, given (1) the sequential development of resistance in the QRDR, in which the *gyrA* S83L mutation is a first step [12], and (2) the concurrent high prevalence of azithromycin resistance in MSM-associated shigellosis, which may increase the therapeutic use of ciprofloxacin, leading to a detrimental "Catch 22" situation in which ciprofloxacin use further drives resistance in this population. Future work should closely monitor the

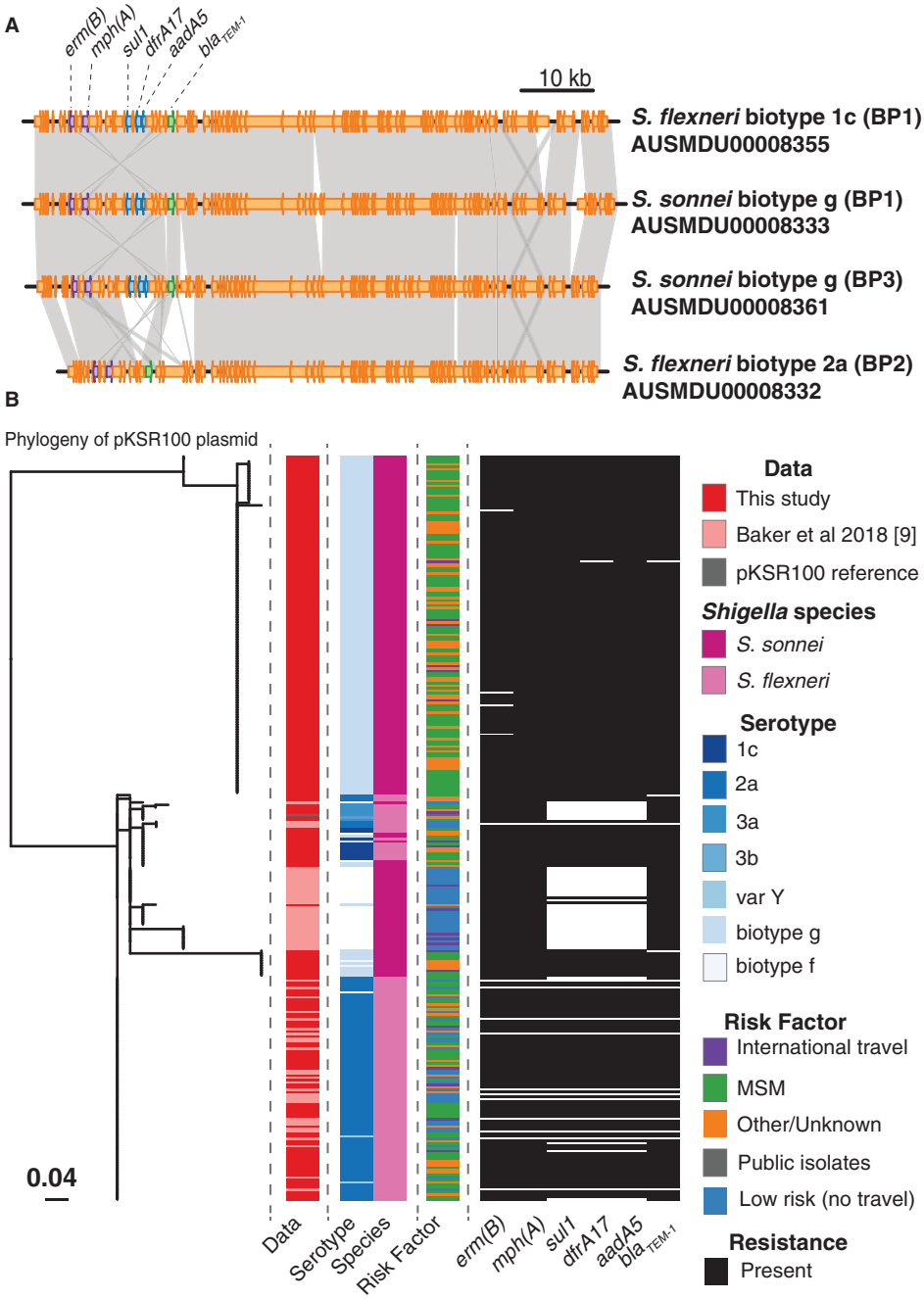

Figure 4. Dissemination of multidrug-resistant plasmid within and between *Shigella* spp. *A,* Comparisons of plasmids detected in 4 Australian *Shigella* isolates. Plasmid genes are shown in orange, with genes mediating resistance to different drug classes highlighted in green (bla_{TEM-1}), purple (*mphA* and *ermB*), and blue (*dfrA17*, *sul1*, and *aadA*). The shaded gray indicates sequence homology regions of ≥95%. *B,* The inferred phylogeny of pKSR100-like plasmids within *Shigella flexneri* and *Shigella sonnei* of isolates that had ≥90% of nucleotide coverage to the pKSR100 reference. The primary risk factor, the species membership, and the serotypes for the isolates are shown to the right of the phylogeny. The *Shigella* species of an isolate is plotted to the right of the phylogeny. A binary heatmap illustrates the presence/absence of 6 genes known to be mobilized on the pKSR100-like plasmids. Abbreviations: BP, Bayesian Analysis of Population Structure group; MSM, men who have sex with men.

clinical outcomes associated with such cases and, indeed, recent guidelines suggest that antimicrobial treatment should now only be reserved for high-risk populations (eg, immunocompromised patients; the very young or elderly; and individuals at risk of causing outbreaks, such as food handlers or childcare workers) [21].

Given our finding of several travel-associated cases within major MSM-associated *S. sonnei* and *S. flexneri* lineages, it is plausible that MSM lineages were imported into Australia, with subsequent onwards transmission in the context of a successful epidemiological triad of host (dense MSM networks with high-risk sexual behaviors), pathogen (highly infectious

bacterial species with transmissible resistance determinants), and environment (selection pressure from azithromycin use). Indeed, many isolates in the Australian *S. flexneri* 2a MSM-associated lineage were highly related at a core genome level to isolates from a previous UK study [10], further highlighting the importance of global travel in propagating shigellosis outbreaks. Moreover, the hypothesis of the importation and domestic spread of pathogens in MSM is also supported by recent outbreaks of other pathogens, such as hepatitis A virus [40] and azithromycin-resistant *Neisseria gonorrhoeae* [41], demonstrating the need for an improved understanding of the factors that may promote such outbreaks, such as human immunodeficiency virus (HIV) co-infection, HIV pre-exposure prophylaxis (PrEP), asymptomatic carriage, and circumstantial features, such as social networking applications and recreational drug use.

In addition to MSM-associated shigellosis, the other major burden of disease in our study was amongst returning travelers. Previous work from the United Kingdom has demonstrated travel-associated *Shigella* sub-lineages, with distinct phylogeographic associations [9]. Here, we broaden this genomic framework extensively to include isolates from returned travelers in Australia. Like the UK study, we observed similar patterns of triple QRDR mutations in *Shigella* isolates from Southern Central Asia (mainly India, Pakistan, and Nepal), and Southeast Asia (mainly Vietnam and Cambodia), further highlighting these regions as reservoirs of resistant enteric pathogens [12], a situation that has parallels with the global emergence of fluoroquinolone-resistant *Salmonella* Typhi [42]. Our study further demonstrates the utility of genomic surveillance in detecting emerging genotypic AMR patterns, using isolates from returned travelers as a proxy for assessing AMR in other regions.

Key strengths of our study include our contemporary sampling frame (ie, 2016–2018); comprehensive coverage of cultured cases of shigellosis; and the integration of detailed epidemiological data. Further, previous studies have demonstrated high interconnectivity of MSM populations in urban Australia [43], meaning that our findings are likely to have applicability across major cities in Australia. Moreover, the inclusion of epidemiologically relevant international isolates provides additional geographic context [9, 10, 13].

Although we received all cultured isolates in the state during the study, this represented only 43% of shigellosis notifications over the study period. This limitation applies to all WGS-based studies of shigellosis, whereby the increasing use of molecular testing for enteric pathogens reduces the availability of isolates for additional analyses [44]. This situation has marked parallels with the use of molecular testing for *N. gonorrhoeae*, where the reduction of bacterial cultures is compromising the ability to detect multidrug-resistant isolates [45]. In an era of increasing AMR in STIs, it is critical that concerted efforts are made to ensure the continuation of culture-based surveillance.

In conclusion, we present, to our knowledge, the first population-based genomic surveillance of shigellosis in Australia. We demonstrate the global dissemination of a multidrug-resistant plasmid, present across multiple continents and highly associated with MSM. This represents a significant and immediate health threat, not only in Australia, but also globally. Urgent multidisciplinary public health measures are required, including the enhanced contact tracing of multidrug-resistant cases of shigellosis (which could be informed by WGS data), improved antimicrobial stewardship, improved information on the clinical outcomes of resistant shigellosis, and heightened awareness of shigellosis as an STI.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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