J Antimicrob Chemother
https://doi.org/10.1093/jac/dkab377

Journal of Antimicrobial Chemotherapy

Genomic epidemiology of persistently circulating MDR *Shigella sonnei* strains associated with men who have sex with men (MSM) in Belgium (2013–19)

Natalie Fischer[1,2], Margo Maex[2], Wesley Mattheus[2], An Van den Bossche[2], Dieter Van Cauteren[3], Valeska Laisnez[3,4], Naïma Hammami[4] and Pieter-Jan Ceyssens [2]*

[1]*European Programme for Public Health Microbiology (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Gustav III:s Boulevard 40, 169 73 Solna, Sweden;* [2]*Division of Human Bacterial Diseases, Sciensano, Rue Engeland 642, 1180 Uccle, Belgium;* [3]*Service Epidemiology of Infectious Diseases, Sciensano, Rue Ernest Blerot 1, 1070 Anderlecht, Belgium;* [4]*Agentschap Zorg en Gezondheid, Koning Albert-II-laan 35 bus 33, 1030 Brussels, Belgium*

*Corresponding author. E-mail: Pieter-Jan.Ceyssens@sciensano.be

Received 30 March 2021; accepted 22 September 2021

Objectives: *Shigella sonnei* resistant to first-line antibiotics azithromycin and ciprofloxacin are on the rise globally. The aim of this study was to describe the epidemiology of MDR *S. sonnei* in Belgium and to identify origins and circulating clusters through WGS.

Methods: We undertook demographic, temporal and geographical analysis of 930 *S. sonnei* isolates submitted to the Belgian National Reference Centre for *Salmonella* and *Shigella* between 2017 and 2019. Phylogenetic analysis of WGS data, genotyping and identification of genetic markers of antimicrobial resistance was performed on 372 Belgian isolates submitted between 2013 and 2019.

Results: *S. sonnei* was identified in 75% (930/1253) of Belgian *Shigella* isolates submitted between 2017 and 2019. Overall, 7% (69/930) of isolates were resistant to ciprofloxacin alone, 6% (57/930) showed reduced susceptibility to azithromycin alone, and 24% (223/930) exhibited both. Men were at higher risk of carrying a double resistant *S. sonnei* strain, compared with women (risk ratio = 8.6, 95% CI = 5.4–13.9). Phylogenetic analysis revealed four independent Belgian clusters of persistently circulating MDR strains, associated with men who have sex with men (MSM) and of the same genotypes as previously described international MSM-related clades. Belgian isolates carried various incompatibility (Inc)-type plasmids, the SpA plasmid and ESBL genes.

Conclusions: In Belgium, *S. sonnei* isolates from men are much more likely to be resistant to important first-line antibiotics than isolates from women. Multiple co-circulating MDR *S. sonnei* clusters of different genotypes were identified in the MSM community. Further studies on risk groups are needed for targeted prevention, improved clinical and public health management and antimicrobial stewardship in Belgium.

Introduction

Shigella are Gram-negative pathogenic enterobacteria that cause severe dysentery with diarrhoea, fever and stomach cramps.[1] Humans are the main reservoir of *Shigella* and transmission occurs mainly through contaminated food or water via the faecal–oral route. The four subgroups *Shigella flexneri, Shigella sonnei, Shigella dysenteriae* and *Shigella boydii* exhibit differences in global distribution; while *S. flexneri* is the main pathogenic agent in childhood diarrhoea in low- to middle-income countries (LMICs), *S. sonnei* affects the adult population of economically developed countries.[2] In the latter setting, international travel to high endemic regions,[3] as well as sexual transmission via oral–anal contact, especially in men who have sex with men (MSM),[4–8] contributes to infection.

WGS has become a powerful tool for genomic epidemiology of shigellosis and enables monitoring of the emergence and circulation of strains on national and global levels.[4,9,10] Recent studies identified five distinct lineages, of which lineage 3 has become globally dominant through acquisition of genes granting antimicrobial resistance (AMR) and colicin-producing plasmids, giving it a competitive advantage.[9,11,12] Still, the limited genomic diversity of

recent *S. sonnei* makes it difficult to differentiate and track strains for public health surveillance or outbreak investigations.[11] To counteract those difficulties, the free open-source software Mykrobe was recently adapted to enable single-nucleotide variant (SNV)-based genotyping and nomenclature based on WGS data, for improved identification and comparison of *S. sonnei* strains across studies and borders.[13]

Shigellosis is usually self-limiting within a couple of days, but fluoroquinolones, cephalosporins and azithromycin are recommended for the treatment of more severe cases with underlying conditions or to limit transmission.[14] A major concern is the recent development of resistance to those first-line antibiotics, via *de novo* mutations in the chromosome or the acquisition of new mobile genetic elements.[15,16] Studies estimate that fluoroquinolone-resistant *S. sonnei* emerged from a single common ancestor in South Asia through consecutive mutations in *gyrA* (S83L), *parC* (S80I) and *gyrA* (D87G), now designated as genotype 3.6.1.1 (aka CipR),[13] before achieving intercontinental dissemination.[17] Resistance to azithromycin and cephalosporins is mainly plasmid-borne and related to the expression of macrolide resistance genes *erm(B) and mph(A)* genes and ESBL genes, respectively.[18] Recent WGS-based studies in Europe, Australia and North America indicate associations of specific, drug-resistant clonal complexes, characterized through mutations in the chromosomal QRDR and carriage of the pKSR100 plasmid of incompatibility (Inc) type FII, with MSM communities.[8,19–23]

Since 1990, the Belgian National Reference Centre for *Salmonella and Shigella* (NRCSS) has continuously monitored AMR in *Shigella* species.[24] In the last decade, fluoroquinolones and azithromycin have been the recommended first-line treatment, and routine laboratory testing to monitor emergence of reduced susceptibility to azithromycin in *Shigella* was implemented in 2017.

The aim of this study was to characterize *S. sonnei* clusters in Belgium, a densely populated country with active MSM communities. We analysed demographical, temporal and geographical trends, with regard to resistance to ciprofloxacin and reduced susceptibility to azithromycin, in the period from 2017 to 2019. Furthermore, we employed WGS for genomic epidemiology to place Belgian isolates from 2013 to 2019 in the context of internationally described *S. sonnei* lineages and genotypes, and to identify Belgian clusters of public health interest, due to MDR, as well as MSM association.

Materials and methods

Sample and data collection

In Belgium, reporting of shigellosis cases is mandatory in the Flanders region, but not in Wallonia and Brussels. Peripheral clinical laboratories collect *Shigella* isolates from human patients and send them voluntarily to the NRCSS for biochemical typing and serotyping, and assessment of AMR using broth microdilution (Sensititre™, Thermo Scientific). Interpretation of MICs was performed using the EUCAST clinical breakpoint table v11.0. Given the absence of a clinical breakpoint, reduced susceptibility to azithromycin was considered for MIC values $\geq$32 mg/L.[18]

Data on residence, gender, age and travel history of cases are voluntarily provided by the clinical laboratory. Data on local MSM-related outbreaks was acquired through interviews conducted by the Flemish Agency for Care and Health (AZG).

Study population for epidemiological analysis

Shigella isolates received from the beginning of January 2017 until the end of December 2019 were eligible and, amongst them, all samples identified as *S. sonnei* were included in the study population for epidemiological analysis (Figure 1). Strains with an undetermined status of resistance to azithromycin or ciprofloxacin were excluded from this analysis. Cases with missing information on gender were excluded from the analysis of relative risk of carriage of a resistant strain between male and female cases.

Study population for WGS analysis

WGS is not yet routinely performed on all isolates received by the Belgian NRCSS. Therefore, 200 *S. sonnei* isolates submitted between 2017 and 2019 were selected for WGS, proportional to their distribution across the 11 Belgian provinces and with 50% reduced susceptibility to azithromycin (Figure 1). Additionally, unpublished data from 172 Belgian *S. sonnei* isolates received and sequenced between 2013 and 2019 for surveillance purposes, and publicly available data from 192 international isolates of different geographical origins and lineages (15 from Africa, 70 from Asia, 15 from Australia, 17 from Latin America, 5 from North America and 70 from Europe) and several with MSM association, were included in the phylogenetic analysis.[9–11,17,20,25]

Sample processing for WGS

Shigella isolates were cultured overnight in BHI broth (BD) at 37°C. DNA was extracted using an MgC Bacterial DNA Kit™ with a 60 µL elution volume (Atrida, Amersfoort, The Netherlands), following the manufacturer's instructions. Sequencing libraries were constructed using the Illumina Nextera XT DNA sample preparation kit and sequenced on an Illumina MiSeq instrument with a 250 bp paired-end protocol (MiSeq v3 chemistry), according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Trimming of short reads was performed with Trimmomatic (version 0.32). Obtained reads were mapped to *S. sonnei* Ss046 (CP000038.1) and SNVs were identified using default settings (CLC Genomic Workbench 20.0.2, QIAGEN, Hilden, Germany). Neighbour joining trees were constructed with the General Time Reversible Nucleotide substitution model, with the transition ratio set at 2.0, the number of substitution rate categories set at 4 and a gamma distribution parameter of 1.0.

ResFinder (version 4.0) was used to determine the presence of AMR genes,[26] PlasmidFinder (version 2.1) was used to detect plasmid replicons[27] and PointFinder (version 3.1) was used to detect chromosomal mutations that confer AMR.[28] The carriage of plasmid pKSR100 was inferred based on the combined presence of resistance genes *mph(A), erm(B),* $bla_{\text{TEM-1B}}$, *aadA5, dfrA17 and sul1*.[20] The presence of the chromosomal resistance island Tn7/Int2 was inferred based on the combination of resistance genes *aadA1 and dfrA1*.[20] Carriage of the SpA plasmid was determined by the combined presence of resistance genes *sul2, tetA(A), strA* and *strB*.[20]

The Mykrobe software package (v0.9.0+) was used to infer hierarchical genotypes and assign nomenclature for lineage, clade, subclade and genotype.[13]

Statistical analysis and visualization

Analysis was performed using R (version 4.0.3) in R Studio (version 1.3.1093). Visualization was performed using packages ggplot2 (version 3.3.2), sf (version 0.9–6) and ggtree (version 2.4.1).[29] Risk ratios were determined using the epi.2by2 function with method = "cohort.count" in the epiR package (version 1.0–15). Differences in proportions between groups were compared by two proportions z-test using the prop.test() function in the stats package (version 4.0.3), which incorporates Yates continuity correction.

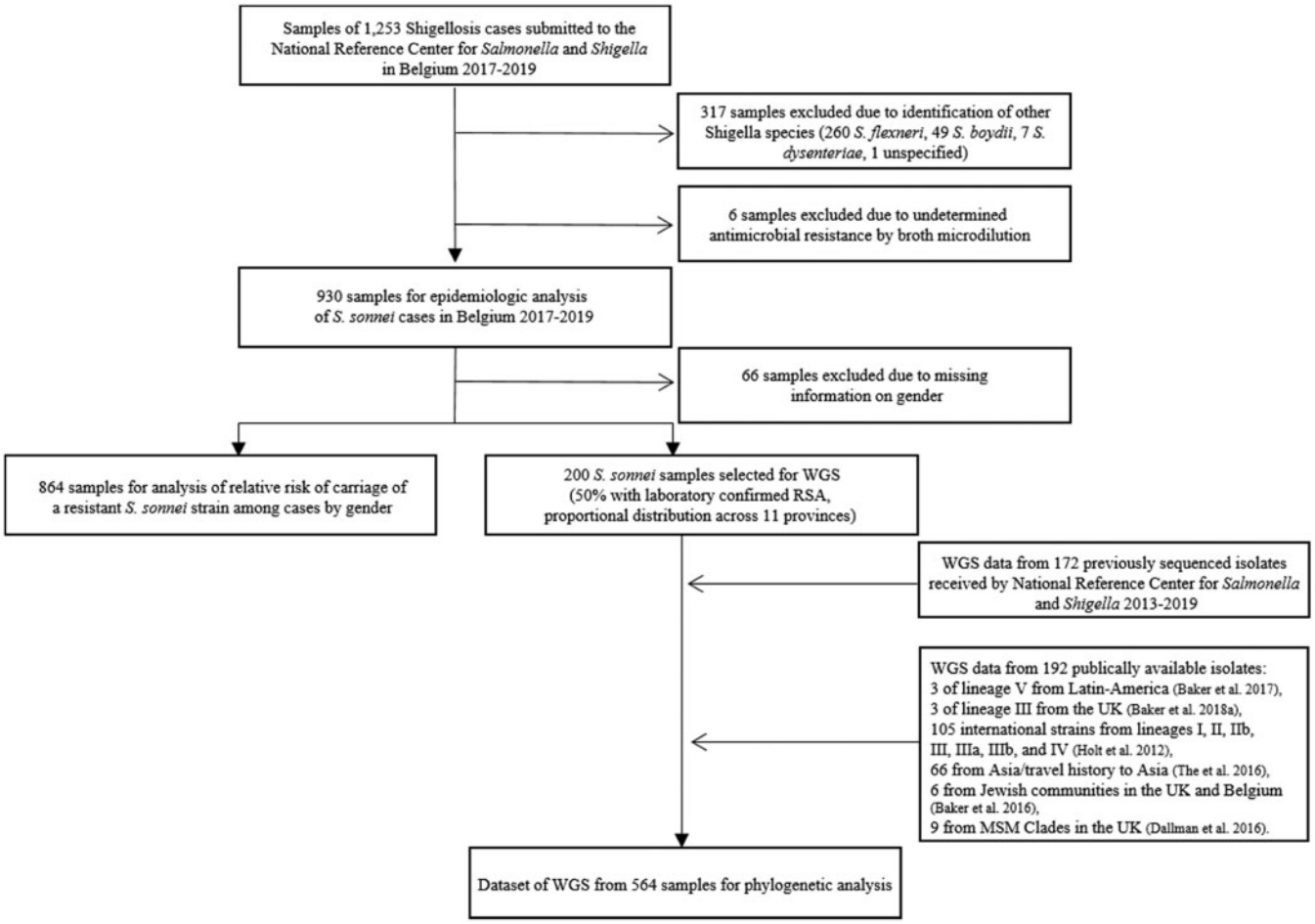

Figure 1. Flow chart of sample selection. Selection of *S. sonnei* isolates submitted to the NRCSS in Belgium between 2017 and 2019 for epidemiological analysis. Selection of *S. sonnei* isolates submitted to the NRCSS in Belgium between 2013 and 2019 for WGS and phylogenetic analysis, as well as integration of publicly available data from international isolates.

Data availability

All WGS sequences have been deposited at the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under Project ID PRJEB40097.

Results

Epidemiological analysis

Amongst the 1253 *Shigella* samples received by the Belgian NRCSS between 2017 and 2019, 75% (936/1253) were identified as *S. sonnei*, 21% (260/1253) as *S. flexneri*, 4% (49/1253) as *S. boydii*, 1% (7/1253) as *S. dysenteriae*, and one sample was unspecified (Figure 1). Of *S. sonnei* strains with conclusive laboratory determination of susceptibility to azithromycin and ciprofloxacin (99%, 930/936), more than half (57%, 514/900 with known age) stemmed from adults aged 25–64 years (Table S1, available as Supplementary data at *JAC* Online). With 57% (493/864 with known gender) a significantly higher proportion of isolates came from men, versus 43% (371/864) from women (Table S1; $P < 0.0001$). Reduced susceptibility to azithromycin alone was determined in 6% (57/930), resistance to ciprofloxacin alone in 7% (69/930), and a combination of both in 24% (223/930) of isolates.

Mapping of *S. sonnei* isolates based on patient postal codes revealed patterns of consistent geographical hot spots, in terms of cases, as well as double resistance, across all 3 years, especially in the two most populated cities Brussels and Antwerp (Figure S1A and B).

The year 2018 was characterized by a rise in isolates from men, compared with the previous year [63% (198/315 with known gender) versus 52% (119/231 with known gender); $P = 0.01$] (Figure 2 and Table S1). Especially, in men in the age group of 25–64 years, the proportion of cases was higher, compared with both the previous year and the following year [67% (132/193 with known age) in 2018 versus 44% (52/117 with known age) in 2017 ($P < 0.0001$) and 56% (98/171 with known age) in 2019 ($P = 0.04$)] (Table S2). Moreover, a significantly higher proportion of infections with double resistant strains was recorded in men, as compared with the previous year and the following year [53% (105/198) in 2018 versus 26% (31/119) in 2017 and 31% (55/176) in 2019; $P < 0.0001$] (Figure 2 and Table S2).

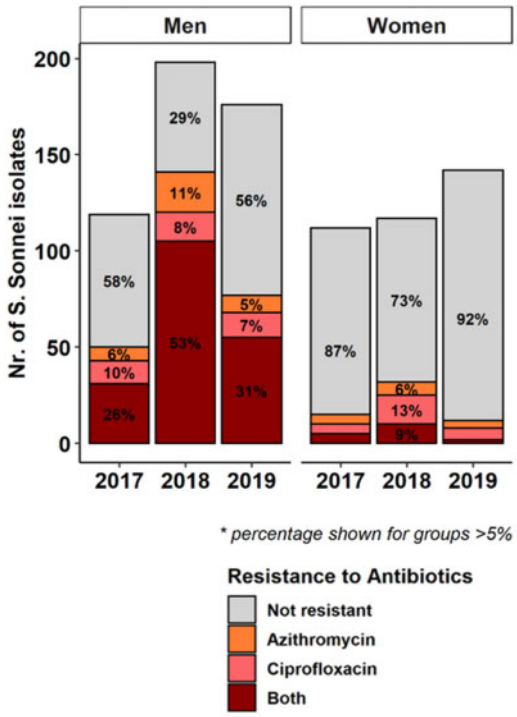

Figure 2. Distribution of AMR in *S. sonnei* per gender and year in Belgium between 2017 and 2019. Number and proportion of *S. Sonnei* isolates submitted to the NCRSS in Belgium between 2017 and 2019, without laboratory confirmed resistance to ciprofloxacin or azithromycin (grey bars), with resistance to ciprofloxacin (orange bars) or azithromycin (pink bars) or combined resistance to ciprofloxacin and reduced susceptibility to azithromycin (dark red bars).

The relative risk of carrying an *S. sonnei* strain with reduced susceptibility to azithromycin alone (RR = 5.1, 95% CI = 3.6–7.2; $P < 0.001$), resistance to ciprofloxacin alone (RR = 4.0, 95% CI = 3.0–5.4; $P < 0.001$) and especially double resistance (RR = 8.6, 95% CI = 5.4–13.9; $P < 0.001$) was higher in male cases across all 3 years, compared with female cases (Table S3).

Phylogenetic analysis

Phylogenetic analysis was performed using 3589 informative SNPs. Clustering with international samples, and genotype analysis using the Mykrobe tool, confirmed that all Belgian *S. sonnei* strains belonged to lineage 3. We identified 27 different genotypes among the Belgian isolates, of which most cases were caused by descendants of the South Asian lineage (12.6% of 3.6.1 aka CipR parent, 11.6% of 3.6.1.1 aka CipR and 27.4% of 3.6.1.1.1.2 aka CipR.MSM5) and genotype 3.7.25 (11.6%, aka MSM4) (Table S4).

Through incorporation of patient data, AMR profiles and designation of genotypes, we identified five independent Belgian clusters of public health interest; four clusters were associated with MSM (designated BEL-I, II, III and IV throughout the manuscript) and one cluster showed combined resistance to ciprofloxacin and reduced susceptibility to azithromycin without MSM association (designated BEL-V throughout the manuscript) (Figure S2). Two Belgian ciprofloxacin-resistant isolates from cases that identified as MSM fell outside those larger clusters. We further characterized each of the clusters in terms of possible origins and acquisition of additional AMR markers.

The largest cluster, BEL-I, was defined as all isolates clonal to or originating from an *S. sonnei* strain first detected in Brussels in 2014, with travel history to China (Figure 3). It contained 102 Belgian isolates, of which the majority clustered <5 SNPs apart, spanned all 11 provinces of Belgium across 5 years (2014–19) and belonged to genotype 3.6.1.1.2 (aka CipR.MSM5). Among BEL-I, 94% (98/104) of isolates came from men, amongst which MSM association could be confirmed by interview in 17% (18/98) (Table S5). Isolates of this clone were of the same genotype as the previously described UK MSM clade 7.[10] Our results indicate that the Belgian 2014 clone acquired the IncFII pKRS100 plasmid carrying *erm(B) and mph(A)*, providing macrolide resistance around 2017, along with a variety of other plasmids including Inc groups P2, B/O/K/Z, I1 and I2, and subsequently spread over the entire country. A sublineage of four isolates from 2018 to 2019 carried the chromosomal island Tn7/Int2, governing *aadA1 and dfrA1* genes, providing resistance to aminoglycosides and trimethoprim. No strain in this cluster carried ESBL genes, but carriage of the small SpA plasmid governing resistance to the second-line antibiotic co-trimoxazole was common. The triple *gyrA* (S83L), *gyrA* (D87G) and *parC* (S80I) mutations in the QRDR among isolates in this cluster conveyed high resistance to ciprofloxacin in the laboratory testing (mean MIC = 6.0 mg/L, SD = 3.5 mg/L).

The closest Belgian isolates in our dataset outside the defined cluster stemmed from 2013, with travel history to India, were 8 SNPs apart from the clone of 2014 and belonged to genotype 3.6.1.1 (aka CipR). The closest foreign isolates in our dataset from Bhutan and India in 2011 were 5 SNPs apart from the 2014 clone and also belonged to genotype 3.6.1.1 (aka CipR).

The BEL-II cluster consisted of 21 Belgian *S. sonnei* isolates (<5 SNPs apart), circulating from 2018 to 2019, that belonged to genotype 3.7.29.1.4.1 (aka VN2.KH1.Aus) (Figure 4). All but one isolate came from men, amongst which MSM association could be confirmed by interview in 29% (6/21) (Figure 4 and Table S5). All isolates carried the single *gyrA* (S83L) mutation, which bestowed low-level resistance to ciprofloxacin in the laboratory testing (mean MIC = 0.25 mg/L, SD = 0.92 mg/L), as well as azithromycin resistance genes *erm(B) and mph(A)*. Furthermore, they possessed the chromosomal resistance island Tn7/Int2, the SpA plasmid, IncB/O/K/Z and IncFII plasmids and ESBL gene $bla_{\text{CTX-M-27}}$.

The BEL-III cluster consisted of six Belgian isolates from 2015 to 2018 (19–23 SNPs apart from clones in BEL-II), which all came from men, but lacked information on sexual orientation (Figure 4 and Table S5). Nevertheless, they were of the same genotype (genotype 3.7.29.1.2 aka VN2.MSM2) as the previously described UK MSM clade 2 from 2016 (1–4 SNPs difference). Belgian isolates carried the single *gyrA* (S83L) mutation, the Tn7/Int2 island and IncFII or IncFIB plasmids. All but one carried the SpA plasmid, one carried both *erm(B) and mph(A)* genes and one isolate carried *mph(A)* and ESBL gene $bla_{\text{DHA-1}}$.

The closest foreign isolates in our dataset stemmed from Vietnam in the early 2000s (15–18 SNPs difference to BEL-II and 6–10 SNPs difference to BEL-III), which initially carried few resistance markers and belonged to genotype 3.7.29.1 (aka VN2). No Belgian case in BEL-II or -III indicated travel to Asia.

BEL-IV was defined as a cluster of six Belgian isolates from 2017 to 2018 with reduced susceptibility to azithromycin, and one

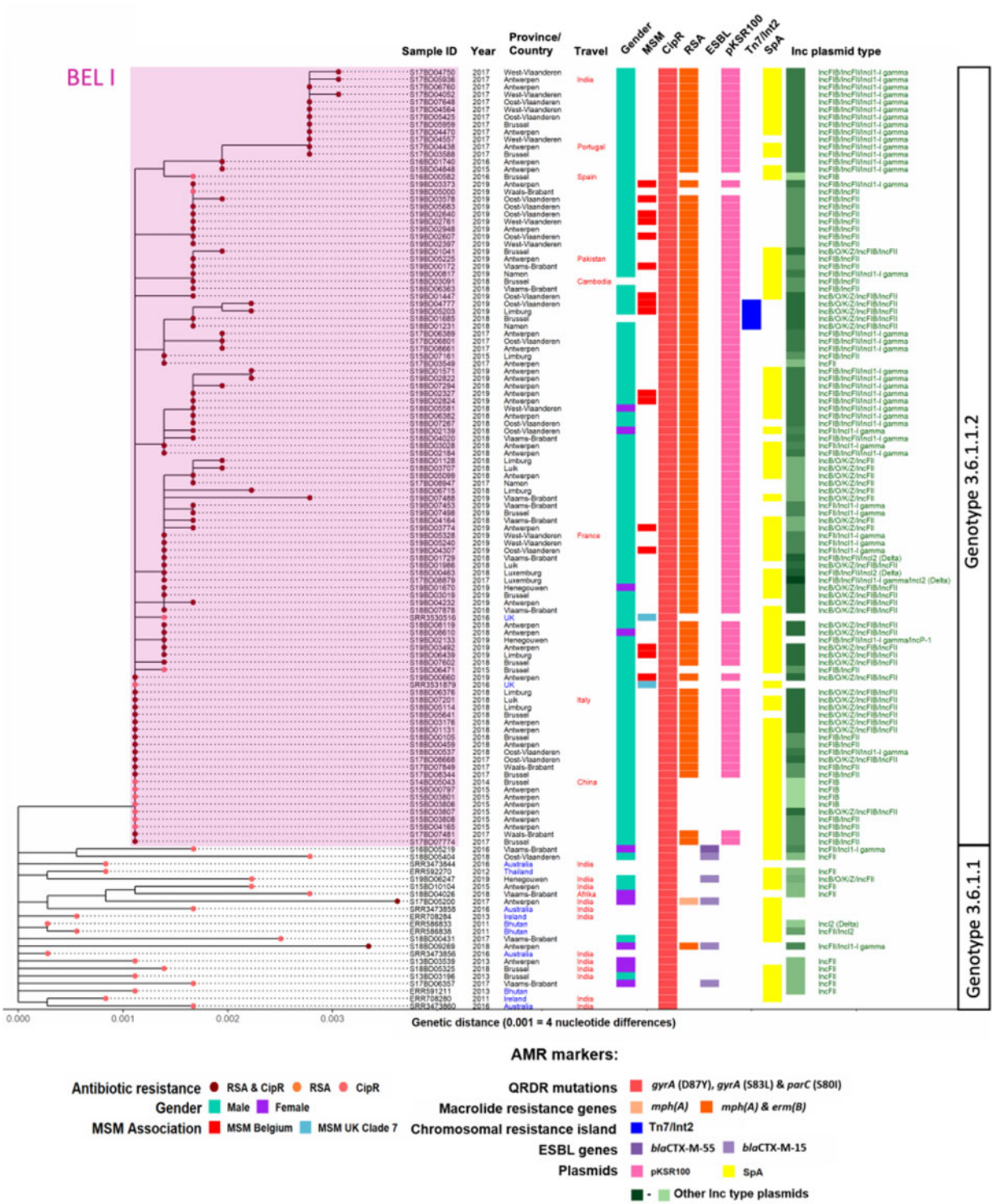

Figure 3. Phylogenetic, temporal, geographical, demographic and AMR characteristics of the BEL-I *S. sonnei* cluster of interest and related isolates. Partial representation of the maximum likelihood phylogenetic tree constructed from WGS data of 372 Belgian and 192 international *S. sonnei* isolates. Isolates belonging to the designated BEL-I cluster of interest are highlighted by pink shading. Isolates are labelled with sample ID, year and Belgian province of isolation (black text label), country of origin for international isolates (blue text label) and, where known, travel history (red text label). The presence of known mutations in *gyrA and parC* genes, which confer resistance to ciprofloxacin (pink circle at the branch tip and squares in the heatmap), the presence of macrolide resistance genes *mph(A) and erm(B)*, which confer reduced susceptibility to azithromycin (orange circle at the branch tip and squares in the heatmap), or a combination of both (dark red circle at the branch tip) are indicated in the phylogenetic tree, as well

Continued

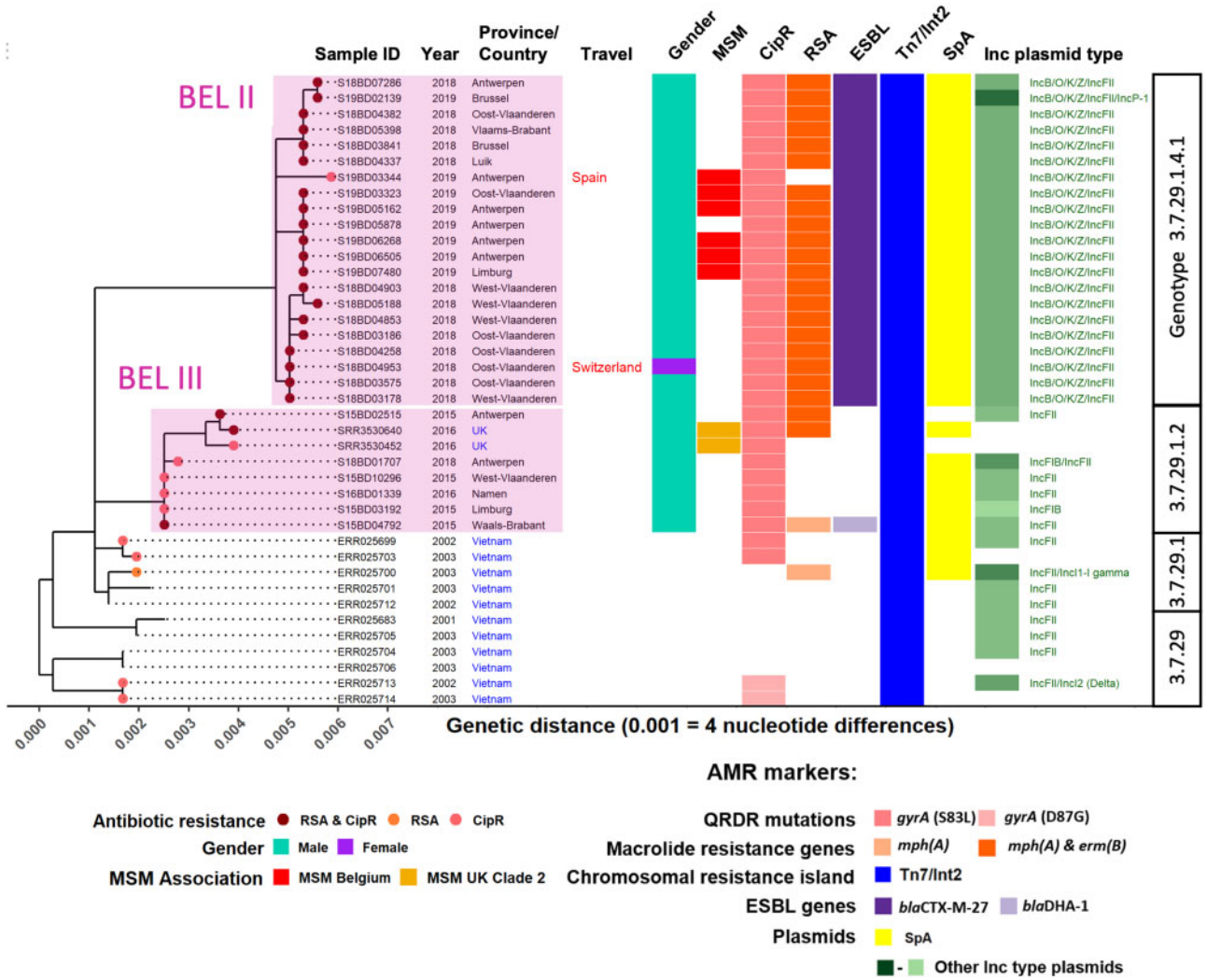

Figure 4. Phylogenetic, temporal, geographical, demographic and AMR characteristics of the BEL-II and BEL-III *S. sonnei* cluster of interest and related isolates. Partial representation of the maximum likelihood phylogenetic tree constructed from WGS data of 372 Belgian and 192 international *S. sonnei* isolates. Isolates belonging to the designated BEL-II and BEL-III clusters of interest are highlighted by pink shading. Isolates are labelled with sample ID, year and Belgian province of isolation (black text label), country of origin for international isolates (blue text label) and, where known, travel history (red text label). The presence of known mutations in the *gyrA* gene, which confer resistance to ciprofloxacin (pink circle at the branch tip and squares in the heatmap), the presence of macrolide resistance genes *mph(A) and erm(B)*, which confer reduced susceptibility to azithromycin (orange circle at the branch tip and squares in the heatmap), or a combination of both (dark red circle at the branch tip) are indicated in the phylogenetic tree, as well as heatmap. Furthermore, the heatmap reflects information on gender, where available, (purple = female, turquoise = male), MSM association [red = Belgian MSM, yellow = UK MSM clade 2 as described by Dallman *et al.*[10] (2016)], presence of ESBL genes (shades of purple), chromosomal resistance island Tn7/Int2 (blue), SpA plasmid (yellow) and Inc plasmids (shades of green, denoted with green text label). The genotype as determined by Mykrobe analysis is denoted in the vertical bars to the right of the heatmap. RSA, reduced susceptibility to azithromycin.

Figure 3. *Continued*
as heatmap. Furthermore, the heatmap reflects information on gender, where available, (purple = female, turquoise = male), MSM association [red = Belgian MSM, light blue = UK MSM clade 7 as described by Dallman *et al.*[10] (2016)], presence of ESBL genes (shades of purple), pKSR100 plasmid (pink), chromosomal resistance island Tn7/Int2 (blue), SpA plasmid (yellow) and Inc plasmids (shades of green, denoted with green text label). The genotype as determined by Mykrobe analysis is denoted in the vertical bars to the right of the heatmap. RSA, reduced susceptibility to azithromycin.

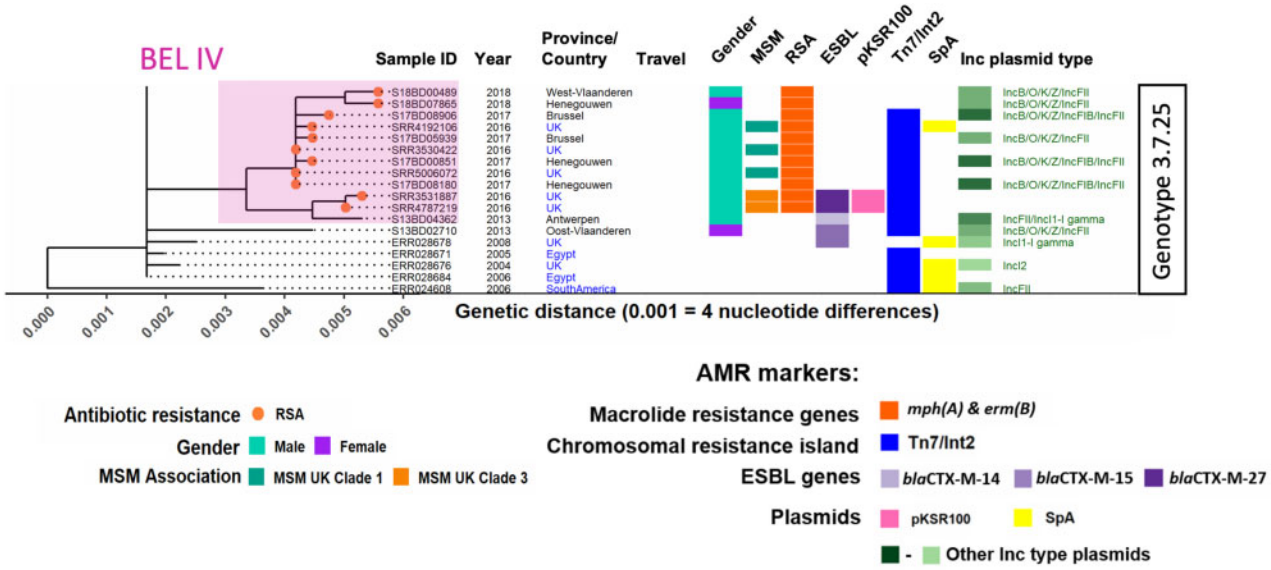

Figure 5. Phylogenetic, temporal, geographical, demographic and AMR characteristics of the BEL-IV *S. sonnei* cluster of interest and related isolates. Partial representation of the maximum likelihood phylogenetic tree constructed from WGS data of 372 Belgian and 192 international *S. sonnei* isolates. Isolates belonging to the designated BEL-IV cluster of interest are highlighted by pink shading. Isolates are labelled with sample ID, year and Belgian province of isolation (black text label), country of origin for international isolates (blue text label) and, where known, travel history (red text label). The presence of macrolide resistance genes *mph(A) and erm(B)*, which confer reduced susceptibility to azithromycin (orange circle at the branch tip and squares in the heatmap), is indicated. Furthermore, the heatmap reflects information on gender, where available, (purple = female, turquoise = male), MSM association [dark turquoise = UK MSM clade 1, orange = UK MSM clade 2 as described by Dallman *et al.*[10] (2016)], presence of ESBL genes (shades of purple), pKSR100 plasmid (pink), chromosomal resistance island Tn7/Int2 (blue), SpA plasmid (yellow) and Inc plasmids (shades of green, denoted with green text label). The genotype as determined by Mykrobe analysis is denoted in the vertical bar to the right of the heatmap. RSA, reduced susceptibility to azithromycin.

Belgian isolate from 2013 without reduced susceptibility to azithromycin, all less than 15 SNPs apart and clustering with isolates from two previously described MSM clades from the UK in 2016 of the same genotype (genotype 3.7.25 aka MSM4) (Figure 5).[10] All but one Belgian isolate came from men, but information on sexual behaviour was not available (Table S5). All isolates in BEL-VI carried *mph(A) and erm(B)* genes, one carried the SpA plasmid and all but two carried the Tn7/Int2 island. Belgian isolates carried IncB/O/K/Z, IncFIB and IncFII plasmids, but not the pKSR100 plasmid, which was found in two UK isolates together with $bla_{\text{CTX-M-27}}$.The closest Belgian isolate outside the defined cluster was 19 SNPs apart and stemmed from 2013. The closest foreign isolate stemmed from Egypt in 2006 (9 SNPs difference). None of the closely relates isolates outside BEL-IV carried macrolide resistance genes, but all were identified as the same genotype (genotype 3.7.25 aka MSM4).

The BEL-V cluster was defined as a branch of 11 identical isolates from the same city in the province of Henegouwen, all belonging to subclade 3.6.3 (Figure S3). All isolates carried the *mph(A)* gene, the *gyrA* (D87Y) mutation and IncFII, as well as other Inc-type plasmids, and all but one carried the $bla_{\text{CTX-M-15}}$ gene. Carriage of the SpA plasmid was also common. Contrary to the other Belgian clusters with double resistance, BEL-V had no MSM association and more than half of the isolates stemmed from children <15 years of age. Isolates in BEL-V were 16–20 SNPs apart from two isolates from 2018 to 2019 outside the cluster with an equal resistance profile, but from other parts of Belgium. The closest foreign isolates in our dataset stemmed from Central Asia (14 SNPs difference) in 2003, did not carry *mph(A)* yet and also belonged to subclade 3.6.3.

Discussion

The integration of WGS into national and international surveillance proves of immense value to elucidate epidemiological origins, identify regional epidemic sublineages and inform antibiotic treatment, especially for *S. sonnei*, where subtyping was previously impossible.[3,9,13,19] Moreover, it allows for discovery of plasmids and other mobile elements crucial for the dissemination of AMR, specially through the use of new long-read sequencing technologies.[15,30] The variety of Inc plasmids we detected in MDR *S. sonnei* isolates points at the flexibility to sustainably take up a wide variety of horizontally acquired genetic material.

S. sonnei was identified in 75% of *Shigella* isolates submitted to the NRCSS in Belgium between 2017 and 2019. We found that one out of three *S. sonnei* isolates presented at least one resistance to first-line antibiotics azithromycin and ciprofloxacin, and one out of four isolates presented with resistance to both. The increase in submitted isolates in 2018 was most likely related to an expansion of MDR clusters in MSM, as the increase occurred mainly among adult men aged 25–64 years, and also resulted in a higher proportion of resistant strains (>50% of isolates with at least one resistance). Our combined analysis of epidemiological and molecular data identified MSM as a risk group for infection with MDR *S. sonnei* in Belgium, similar to what has been observed in other countries.[4,8,19,21–23] We identified several independent continuously

circulating multiresistant clusters of different genotypes and with different AMR profiles in Belgium, linked to MSM behaviour, and of the same genotypes as known international MSM-related clades.[13]

The largest MSM-related cluster we observed in Belgium (BEL-I), consisted of isolates of ciprofloxacin- and azithromycin-resistant genotype 3.6.1.1.2 (aka CipR.MSM5), which expanded especially in the years 2018 and 2019. Similarly, the same genotype was also responsible for the dissemination and subsequent increase in ciprofloxacin- and azithromycin-resistant *S. sonnei* in Australia and England, especially through MSM-related outbreaks, and the USA, over the last couple of years.[13]

Furthermore, we identified two Belgian MSM-related clusters, originating from *S. sonnei* strains in Vietnam (genotype 3.7.29 aka Vietnam III).[11,31] Isolates of BEL-II belonged to the same genotype (genotype 3.7.29.1.2 aka VN2.MSM2) as previously described MSM-related strains in the UK.[19] Isolates of BEL-III belonged to genotype 3.7.29.1.4.1 (aka VN2.KH1.Aus), which had been circulating in the Australian MSM community between 2016 and 2018, and was later responsible for a prolonged outbreak in men in the state of Victoria in 2019.[22,32] The additional carriage of ESBL genes, such as $bla_{\text{CTX-M-27}}$, could present an advantage for the rapid expansion and dissemination of this MDR *S. sonnei*, as observed during the outbreak in Australia, our study in Belgium and recently reported in Switzerland.[33]

It should be noted, that outbreaks of MDR *S. sonnei* carrying ESBL genes were not limited to the MSM community in Belgium, but also observed in a different demographic altogether, in children and young adults in cluster BEL-V (carrying $bla_{\text{CTX-M-15}}$). Overall, carriage of ESBL genes was reported to still be relatively low among global *S. sonnei* isolates (median 17.2%) by Hawkey *et al.*,[13] but worldwide antibiotic stewardship is pertinent in any given patient population.

The rise in AMR in Asia, where many of the currently circulating MDR *S. sonnei* strains emerged and antimicrobial stewardship is challenging, is of great concern.[17,34] Like the burden of AMR, the means to perform high-technology surveillance using WGS is also disproportionally distributed. Collaboration and exchange of expertise between high-income countries and LMICs, and access to open-source analysis tools and sharing of sequencing data will be crucial to successfully combat AMR globally.[35]

Our analysis was limited by different requirements for reporting of shigellosis and voluntary sample submission in the Belgian regions. Interviews on sexual behaviour were only conducted in cases from Flanders, which limited our ability for risk assessment of AMR in the broader Belgian MSM community. Furthermore, WGS was not yet routinely integrated in the surveillance of shigellosis in Belgium and data availability was therefore not exhaustive. Our dataset included data from international studies, representative of different known *S. sonnei* lineages, but did not include all available global datasets. This limitation was counteracted by the incorporation of genotype determination through the recently published Mykrobe pipeline for *S. sonnei*, which allowed for comparison with international isolates. Nevertheless, this genotype assignment pipeline does not currently include mobile elements, such as the diverse collection of Inc-type plasmids found in our Belgian isolates.

Our findings highlight the importance of spread of multiresistant *S. sonnei* strains in the MSM community and the necessity to implement specific control measures and increased surveillance in Belgium. More specifically, we showed that Belgian MSM-associated strains were of the same genotypes as previously described MSM-associated clades in the UK, Switzerland, Australia and the USA.[8,10,13,19,22,32] These findings highlight the public health relevance of the connectivity of the MSM community in Europe and beyond. This poses challenges for effective prevention and control, but also offers opportunities for network-based epidemiological research and targeted interventions, even where MSM behaviour is stigmatized.[36]

Based on our results, we recommend routine countrywide reporting of shigellosis cases and submission of isolates to the NRCSS, the integration of questionnaires on sexual behaviour and routine WGS in *Shigella* surveillance in Belgium. This will be crucial for future research on risk groups, needed to develop targeted prevention campaigns and guidelines for appropriate clinical and public health management.[37] In light of the globally observed rise in resistance to ciprofloxacin and azithromycin, as well as the threat of increased exchange and carriage of ESBL genes, the treatment guidelines for shigellosis should be re-evaluated and AMR profiling should be employed for improved antibiotic stewardship.[5,7,17]

Acknowledgements

We thank the technical staff from the Belgian NRCSS for their excellent technical assistance. We thank the Belgian clinical laboratories that submitted isolates and reported cases, as well as the doctors, who provided information on risk factors and helped establish case management measures. We would like to thank Henri George for his help with the python code development.

Funding

The NRCSS and this work was partially supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System.

N.F. is a fellow in the European Public Health Microbiology Programme funded through the European Programme for Intervention Epidemiology Training, European Centre for Disease Prevention and Control.

Transparency declarations

None to declare.

Access to data

All authors had full access to the data. P.-J.C. is the guarantor for the data.

Author contributions

Conception and design of the study: N.F., W.M., A.V.d.B. and P.-J.C. Acquisition of data: N.F., W.M., A.V.d.B., D.V.C., V.L., N.H. and P.-J.C. Data analysis: N.F. and M.M. Interpretation of data: N.F. and P.-J.C. Drafting of article: N.F. Revision and final approval of the version: all authors revised and approved the final manuscript version.

Supplementary data

Tables S1 to S5 and Figures S1 to S3 are available as Supplementary data at *JAC* Online.

References

1 Sansonetti PJ. *Pathogenesis of Shigellosis*. Springer-Verlag Berlin Heidelberg, 1992.

2 Anderson M, Sansonetti PJ, Marteyn BS. *Shigella* diversity and changing landscape: insights for the twenty-first century. *Front Cell Infect Microbiol* 2016; **6**: 45.

3 Van den Bossche A, Ceyssens P-J, Denayer S *et al*. Outbreak of Central American born *Shigella sonnei* in two youth camps in Belgium in the summer of 2019. *Eur J Clin Microbiol Infect Dis* 2021; **40**: 1573–7.

4 Mitchell HD, Mikhail AFW, Painset A *et al*. Use of whole-genome sequencing to identify clusters of *Shigella flexneri* associated with sexual transmission in men who have sex with men in England: a validation study using linked behavioural data. *Microb Genom* 2019; **5**: e000311.

5 Baker KS, Dallman TJ, Ashton PM *et al*. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. *Lancet Infect Dis* 2015; **15**: 913–21.

6 Gaudreau C, Ratnayake R, Pilon PA *et al*. Ciprofloxacin-resistant *Shigella sonnei* among men who have sex with men, Canada, 2010. *Emerg Infect Dis* 2011; **17**: 1747–50.

7 Chiou C-S, Izumiya H, Kawamura M *et al*. The worldwide spread of ciprofloxacin-resistant *Shigella sonnei* among HIV-infected men who have sex with men, Taiwan. *Clin Microbiol Infect* 2016; **22**: 383.e11–6.

8 Hinic V, Seth-Smith H, Stöckle M *et al*. First report of sexually transmitted multi-drug resistant *Shigella sonnei* infections in Switzerland, investigated by whole genome sequencing. *Swiss Med Wkly* 2018; **148**: w14645.

9 Baker KS, Campos J, Pichel M *et al*. Whole genome sequencing of *Shigella sonnei* through PulseNet Latin America and Caribbean: advancing global surveillance of foodborne illnesses. *Clin Microbiol Infect* 2017; **23**: 845–53.

10 Dallman TJ, Chattaway MA, Mook P *et al*. Use of whole-genome sequencing for the public health surveillance of *Shigella sonnei* in England and Wales, 2015. *J Med Microbiol* 2016; **65**: 882–4.

11 Holt KE, Baker S, Weill F-X *et al*. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. *Nat Genet* 2012; **44**: 1056–9.

12 Thompson CN, Duy PT, Baker S. The Rising Dominance of *Shigella sonnei*: an intercontinental shift in the etiology of bacillary dysentery. *PLoS Negl Trop Dis* 2015; **9**: e0003708.

13 Hawkey J, Paranagama K, Baker KS *et al*. Global population structure and genotyping framework for genomic surveillance of the major dysentery pathogen, *Shigella sonnei*. *Nat Commun* 2021; **12**: 2684.

14 Christopher PR, David KV, John SM *et al*. Antibiotic therapy for *Shigella* dysentery. *Cochrane Database Syst Rev* 2010; issue **8**: CD006784.

15 Baker KS, Dallman TJ, Field N *et al*. Horizontal antimicrobial resistance transfer drives epidemics of multiple Shigella species. *Nat Commun* 2018; **9**: 1462.

16 Torraca V, Holt K, Mostowy S. Shigella sonnei. *Trends Microbiol* 2020; **28**: 696–7.

17 Chung The H, Rabaa MA, Pham Thanh D *et al*. South Asia as a reservoir for the global spread of ciprofloxacin-resistant *Shigella sonnei*: a cross-sectional study. *PLoS Med* 2016; **13**: e1002055.

18 Salah M, Shtayeh I, Ghneim R *et al*. Evaluation of *Shigella* species azithromycin CLSI epidemiological cutoff values and macrolide resistance genes. *J Clin Microbiol* 2019; **57**: e01422-18.

19 Bardsley M, Jenkins C, Mitchell HD *et al*. Persistent transmission of shigellosis in England is associated with a recently emerged multidrug-resistant strain of *Shigella sonnei*. *J Clin Microbiol* 2020; **58**: e01692-19.

20 Baker KS, Dallman TJ, Field N *et al*. Genomic epidemiology of *Shigella* in the United Kingdom shows transmission of pathogen sublineages and determinants of antimicrobial resistance. *Sci Rep* 2018; **8**: 7389.

21 Yousfi K, Gaudreau C, Pilon PA *et al*. Genetic mechanisms behind the spread of reduced susceptibility to azithromycin in *Shigella* strains isolated from men who have sex with men in Québec, Canada. *Antimicrob Agents Chemother* 2019; **63**: e01679-18.

22 Ingle DJ, Andersson P, Valcanis M *et al*. Prolonged outbreak of multidrug-resistant *Shigella sonnei* harboring $bla_{CTX\text{-}M\text{-}27}$ in Victoria, Australia. *Antimicrob Agents Chemother* 2020; **64**: e01518-20.

23 Worley JN, Javkar K, Hoffmann M *et al*. Genomic drivers of multidrug-resistant *Shigella* affecting vulnerable patient populations in the United States and abroad. *mBio* 2021; **12**: e03188-20.

24 Vrints M, Mairiaux E, Meervenne EV. Surveillance of antibiotic susceptibility patterns among *Shigella sonnei* strains isolated in Belgium during the 18-year period 1990 to 2007. *J Clin Microbiol* 2009; **47**: 1379–85.

25 Baker KS, Dallman TJ, Behar A *et al*. Travel- and community-based transmission of multidrug-resistant *Shigella sonnei* lineage among international Orthodox Jewish communities. *Emerg Infect Dis* 2016; **22**: 1545–53.

26 Bortolaia V, Kaas RS, Ruppe E *et al*. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500.

27 Carattoli A, Zankari E, García-Fernández A *et al*. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.

28 Zankari E, Allesøe R, Joensen KG *et al*. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J Antimicrob Chemother* 2017; **72**: 2764–8.

29 Yu G, Smith DK, Zhu H *et al*. GGTREE: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol Evol* 2017; **8**: 28–36.

30 Orlek A, Stoesser N, Anjum MF *et al*. Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. *Front Microbiol* 2017; **8**: 182.

31 Holt KE, Thieu Nga TV, Thanh DP *et al*. Tracking the establishment of local endemic populations of an emergent enteric pathogen. *Proc Natl Acad Sci USA* 2013; **110**: 17522–7.

32 Ingle DJ, Easton M, Valcanis M *et al*. Co-circulation of multidrug-resistant *Shigella* among men who have sex with men in Australia. *Clin Infect Dis* 2019; **69**: 1535–44.

33 Campos-Madueno EI, Bernasconi OJ, Moser AI *et al*. Rapid increase of CTX-M-producing *Shigella sonnei* isolates in Switzerland due to spread of common plasmids and international clones. *Antimicrob Agents Chemother* 2020; **64**: e01057-20.

34 Pierce J, Apisarnthanarak A, Schellack N *et al*. Global antimicrobial stewardship with a focus on low- and middle-income countries: a position statement for the International Society for Infectious Diseases. *Int J Infect Dis* 2020; **96**: 621–9.

35 NIHR Global Health Research Unit on Genomic Surveillance of AMR. Whole-genome sequencing as part of national and international surveillance programmes for antimicrobial resistance: a roadmap. *BMJ Glob Health* 2020; **5**: e002244.

36 Amirkhanian YA. Social networks, sexual networks and HIV risk in men who have sex with men. *Curr HIV/AIDS Rep* 2014; **11**: 81–92.

37 Clutterbuck D, Asboe D, Barber T *et al*. 2016 United Kingdom national guideline on the sexual health care of men who have sex with men. *Int J STD AIDS* 2018; doi:10.1177/0956462417746897.