

Emergence of novel strains of *Shigella flexneri* associated with sexual transmission in adult men in England, 2019–2020

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Abstract

National surveillance of shigellosis in England revealed an increase in sexually transmitted *Shigella flexneri* in adult males in 2019 that persisted throughout 2020. We observed a resurgence of azithromycin-resistant *S. flexneri* serotype 3a, and the emergence of two novel multidrug-resistant clades of *S. flexneri* 2a and *S. flexneri* 1b.

DATA SUMMARY

The authors confirm that all supporting data, code and protocols have been provided within the article or through supplementary data files (one supplementary table).

There is robust evidence that domestically acquired shigellosis in adult males in western industrialized countries is associated with sexual transmission among men who have sex with men (MSM) [1, 2]. In England in 2019, the number of cases of adult males with domestically acquired *Shigella sonnei* infection averaged 20 cases per month, while in 2020, contemporaneous with coronavirus disease 2019 (COVID-19) pandemic measures to promote social distancing (lockdown restrictions), cases declined to fewer than 5 per month (Fig. 1). In comparison, during 2019 there was an increase in monthly *Shigella flexneri* notifications in adult males, from 16 cases in January to 41 in December 2019, and despite a rapid decline in March 2020, numbers quickly returned to pre-COVID-19 lockdown levels by September 2020 (Fig. 1). Here, we describe preliminary data suggesting that the recent increase in *S. flexneri* is driven by a resurgence of *S. flexneri* serotype 3a, and the concurrent emergence of two clades (clade is defined here as a 250-SNP single-linkage cluster) of *S. flexneri*, not previously associated with sexual transmission in MSM in England.

This study included all *S. flexneri* and *S. sonnei* isolates referred to Public Health England (PHE) by hospital laboratories in England between January 2019 and October 2020. Demographic data submitted alongside laboratory

isolates (name, date of birth, sex and foreign travel history) were collated. Genome sequences were clustered based on genomic similarity [3].

Quality- and adapter-trimmed Illumina reads were aligned to the *S. sonnei* reference genome Ss46 (GenBank accession: NC007384.1) or the *S. flexneri* serotype 2a strain 2457T (GenBank accession: AE014073.1) using BWA MEM v0.7.12 [4]. The resulting sequence alignment maps were sorted and indexed to produce binary alignment maps using SAMtools [5]. High-quality variant positions (mapping quality >30, depth >10, variant ratio >0.9) identified using GATK v2.6 in unified genotyper mode [6] were extracted and stored in SnapperDB [7]. Hierarchical single-linkage clustering was performed on the pairwise SNP distance matrix at descending SNP thresholds (250, 100, 50, 25, 10, 5 and 0), as previously described [7]. The clustering is summarized as a ‘SNP address’ (a seven-digit code) that describes the cluster membership at each of the thresholds.

Antimicrobial resistance (AMR) determinants were detected using GeneFinder [8] (https://github.com/phe-bioinformatics/gene_finder), a customized algorithm that utilizes Bowtie 2 [9] to map sequenced reads to a database of reference sequences [5]. Genes were defined as present if they represented 100% of the reference sequence, with greater than 90% nucleotide identity. Phenotypic antimicrobial susceptibility profiles were inferred from the genome-derived data, based on previous validation studies [10].

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Keywords: shigellosis; sexual transmission; surveillance; multidrug resistance.

Abbreviations: AMR, antimicrobial resistance; CC, clonal complex; COVID, coronavirus disease; MSM, men who have sex with men; PHE, Public Health England; SNP, single nucleotide polymorphism; UK, United Kingdom.

FASTQ reads from all sequences in this study can be found at the PHE Bioproject PRJNA315192.

One supplementary table are available with the online version of this article.

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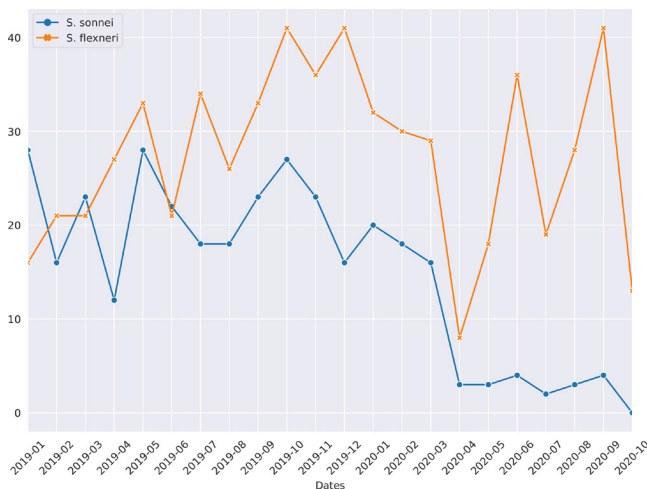


Fig. 1. Trends in *S. flexneri* and *S. sonnei* diagnoses reported in adult males with no history of travel outside the UK.

Clusters were designated as ‘adult male’ where at least 90% were men aged 16 years or older. Use of adult male clusters as a pragmatic proxy for MSM transmission has been validated using detailed questionnaires [2]. A threshold of 10 SNPs difference across the core genome of any 2 isolates is the current standard for defining likely transmission clusters in routine public health surveillance of *Shigella* species at PHE [2].

Between 1 January 2019 and 31 October 2020, the total number of confirmed *S. sonnei* and *S. flexneri* diagnoses in England was 1956, of which 1360 (69.5%) were male, 1677 (85.7%) were aged 16 or over and 1449 (74.0%) were not known to be associated with travel outside the UK (non-travel-associated) (Fig. 2). There were 14 10-SNP single-linkage clusters comprising more than 5 cases and with a male-to-female ratio of >9:1 (Table 1, Figs. 3 and

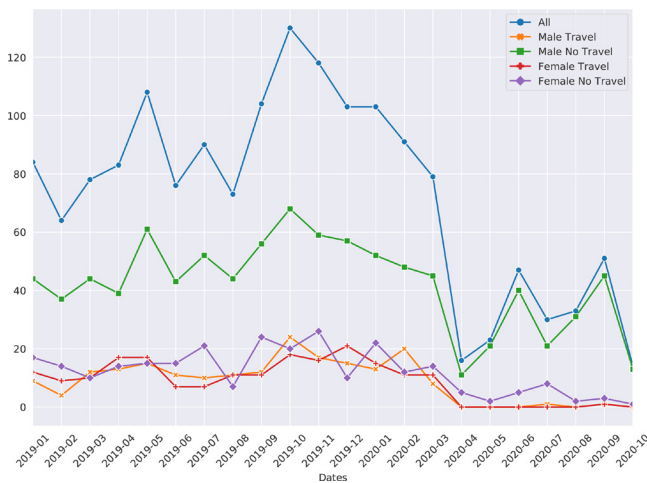


Fig. 2. Trends in shigellosis in adults by sex and travel status.

4). These 14 clusters were subsequently condensed into 8 phylogenetic clusters based on phylogenetic shared ancestry comprising 753 isolates. A further 76 isolates that were direct phylogenetic descendants of these clusters (but not within the 10-SNP threshold) were also included, making a total of 829 isolates, comprising 42.4% (829/1956) of all isolates received in the study period that fitted the criteria for putative MSM transmission.

The largest cluster (216/753, 28.8%; CC152-377 in Table 1, Fig. 3) comprised isolates belonging to *S. sonnei* clade 5 exhibiting multidrug resistance to azithromycin and ciprofloxacin, in addition to harbouring genes known to confer resistance to ampicillin, streptomycin, trimethoprim, sulphonamides, tetracycline and chloramphenicol. During 2018, *S. sonnei* clade 5 was responsible for the highest burden of shigellosis in the MSM community [11] and remained the most common cause of shigellosis circulating within the MSM community until the implementation of lockdown restrictions on 23 March 2020 (Fig. 5). Subsequently, the number of reported cases decreased dramatically and has remained at fewer than five cases per month (Figs. 1 and 5).

In contrast, *S. flexneri* MSM-associated clusters have persisted during the post-lockdown period. Cluster CC245-1189 (Table 1 and Fig. 4) comprised isolates that fell within the previously described *S. flexneri* MSM 3a epidemic clade responsible for the surge in cases of shigellosis between 2009 and 2014 and characterized by the acquisition of a plasmid-encoding resistance to azithromycin [1, 12]. The number of cases caused by this pathogen had decreased to pre-epidemic levels during 2017–2018. However, during 2019 we observed a resurgence that has been maintained throughout the period of lockdown restrictions (Fig. 5). Cases caused by isolates belonging to the previously described azithromycin-resistant *S. flexneri* MSM 2a epidemic clade (including the following 10-SNP single-linkage clusters, CC245-820, CC245-727, CC242-42 and CC245-1458 in Table 1 and Fig. 4) [13], declined in the same period (Fig. 5).

During 2019, a novel clade of *S. flexneri* 2a emerged as a cause of sexually transmitted shigellosis in men in 2018 (including the following 10-SNP single-linkage clusters, CC245-1629, CC245-1599, CC245-1580 in Table 1 and Fig. 4). The novel *S. flexneri* 2a 2020 clade did not evolve directly from the previously described *S. flexneri* 2a epidemic clone and is located on a different branch of the phylogeny (Fig. 4). In addition, two clades of *S. flexneri* belonging to serotypes 1b (specifically the 10-SNP single-linkage cluster CC245-642 in Table 1 and Fig. 4) and 1c (including the 10-SNP single-linkage cluster CC245-397 and CC245-1523 in Table 1 and Fig. 4) were identified. These clades have been associated with sexual transmission among MSM in other countries [14, 15], but they had not been identified in this cohort in England prior to this study (Table 1 and Fig. 5).

Isolates belonging to serotypes 2a and 1b have maintained steady levels of transmission despite the lockdown

Table 1. Demographic characteristics and genome-derived AMR profiles of the 10-SNP single-linkage clusters of genome sequences from all isolates submitted to GBRU from January 2019 to October 2020. Each clade represents a 10-SNP single-linkage cluster. *Shigella* diagnosis confirmed by PHE during the study period

| 10-SNP single-linkage cluster designation | Species/serotype | No. | Male | Adult | Travel | Genome-derived AMR profile | Predicted phenotypic AMR profile [10] |
|---|--------------------------|-----|------|-------|--------|---|---------------------------------------|
| CC245-1684 MSM_3_a_2020 | <i>S. flexneri</i> 3a | 15 | 15 | 15 | 0 | OXA-1/aadA1b/mphA/dfrA5/tetA/sul2/cat1 | AMP/STR/TRM/TET/ SUL/CHL |
| CC245-1629 MSM_2_a_2020 | <i>S. flexneri</i> 2a | 12 | 12 | 12 | 0 | OXA-1/gyrAS83L;D87N;parCS80I/dfrA1/ tetA/cat1 | AMP/CIP/TRM/TET/CHL |
| CC245-1599 MSM_2_a_2020 | <i>S. flexneri</i> 2a | 9 | 9 | 9 | 0 | OXA-1/strAB/gyrAS83L;D87N;parCS80I/ dfrA1/tetA/sul2/cat1 | AMP/STR/CIP/TRM/ TET/SUL/CHL |
| CC245-1580 MSM_2_a_2020 | <i>S. flexneri</i> 2a | 96 | 95 | 96 | 4 | OXA-1/gyrAS83L;D87N;parCS80I/dfrA1/ tetA/sul2/cat1 | AMP/CIP/TRM/TET/ SUL/CHL |
| CC245-1189 MSM-3a epidemic | <i>S. flexneri</i> 3a | 130 | 122 | 129 | 4 | OXA-1/mphA/tetA/cat1 | AMP/TET/CHL |
| CC245-820 MSM-2a epidemic | <i>S. flexneri</i> 2a | 27 | 27 | 27 | 0 | OXA-1/tetA/cat1 | AMP/TET/CHL |
| CC245-727 MSM-2a epidemic | <i>S. flexneri</i> 2a | 47 | 47 | 46 | 4 | TEM-1,OXA 1/aadA1b,aadA5/ermB,mphA/ dfrA17/tetA/sul1/cat1 | AMP/STR/AZM/TRM/ TET/SUL/CHL |
| CC242-42 MSM-2a epidemic | <i>S. flexneri</i> 2a | 45 | 45 | 45 | 2 | TEM-1,OXA 1/aadA1b,aadA5/ermB,mphA/ dfrA17/tetA/sul1/cat1 | AMP/STR/AZM/TRM/ TET/SUL/CHL |
| CC245-1458 MSM-2a epidemic | <i>S. flexneri</i> 2a | 11 | 11 | 11 | 0 | TEM-1,OXA 1/aadA1b,aadA5/ermB,mphA/ dfrA17/tetA/sul1/cat1 | AMP/STR/AZM/TRM/ TET/SUL/CHL |
| CC245-397 MSM_1_c | <i>S. flexneri</i> 1c | 6 | 6 | 6 | 1 | TEM-1/strAB,addA5/qnrS/mphA/ dfrA14,dfrA17/tetA/sul1,sul2 | AMP/STR/TRM/TET/ SUL/CHL |
| CC245-1523 MSM_1_c | <i>S. flexneri</i> 1c | 29 | 28 | 29 | 0 | TEM-1/addA5/mphA/dfrA17/tetA/sul1 | AMP/STR/TRM/TET/SUL |
| CC245-642 MSM_1b | <i>S. flexneri</i> 1b | 104 | 100 | 102 | 4 | OXA-1/strAB/dfrA1/tetA/sul2/cat1 | AMP/STR/TRM/TET/ SUL/CHL |
| CC152-377 MSM_Clade_5 | <i>S. sonnei</i> | 216 | 204 | 215 | 18 | TEM-1/strAB, addA5/ gyrAS83L;D87N;parCS80I/ermB,mphA/ dfrA1,dfrA17/tetA/sul1,sul2 | AMP/STR/CIP/AZM/ TRM/TET/SUL |
| CC152-35 MSM_Clade_2 | <i>S. sonnei</i> | 6 | 6 | 6 | 0 | TEM-1/strAB, addA5/gyrAS83L/ermB,mphA/ dfrA1,dfrA17/tetA/sul1,sul2 | AMP/STR/CIP/AZM/ TRM/TET/SUL |

AMP, ampicillin; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; STR, streptomycin; SUL, sulphonamides; TET, tetracycline; TRM, trimethoprim.

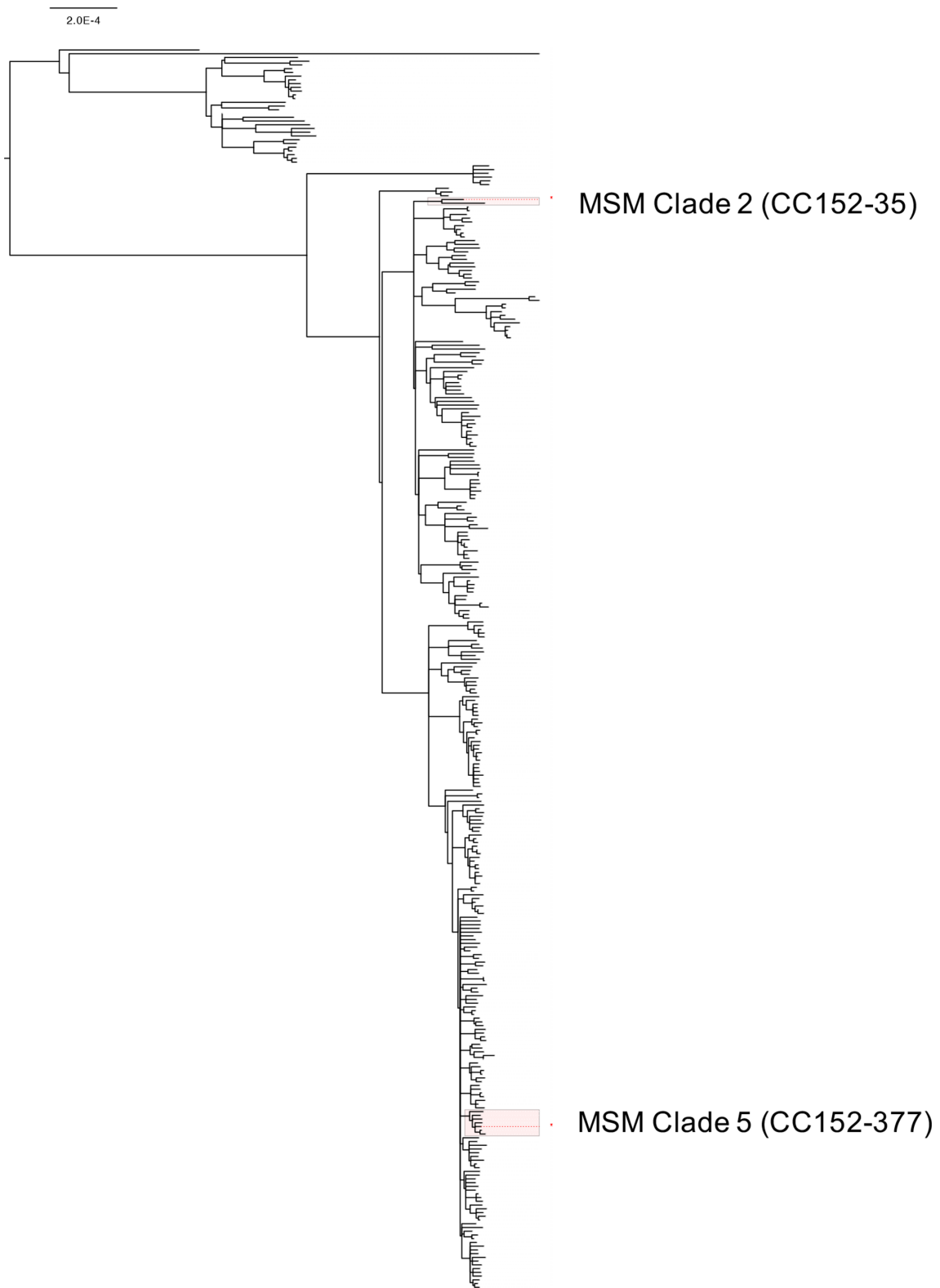


Fig. 3. Population structure of isolates of *S. sonnei* included in this study.

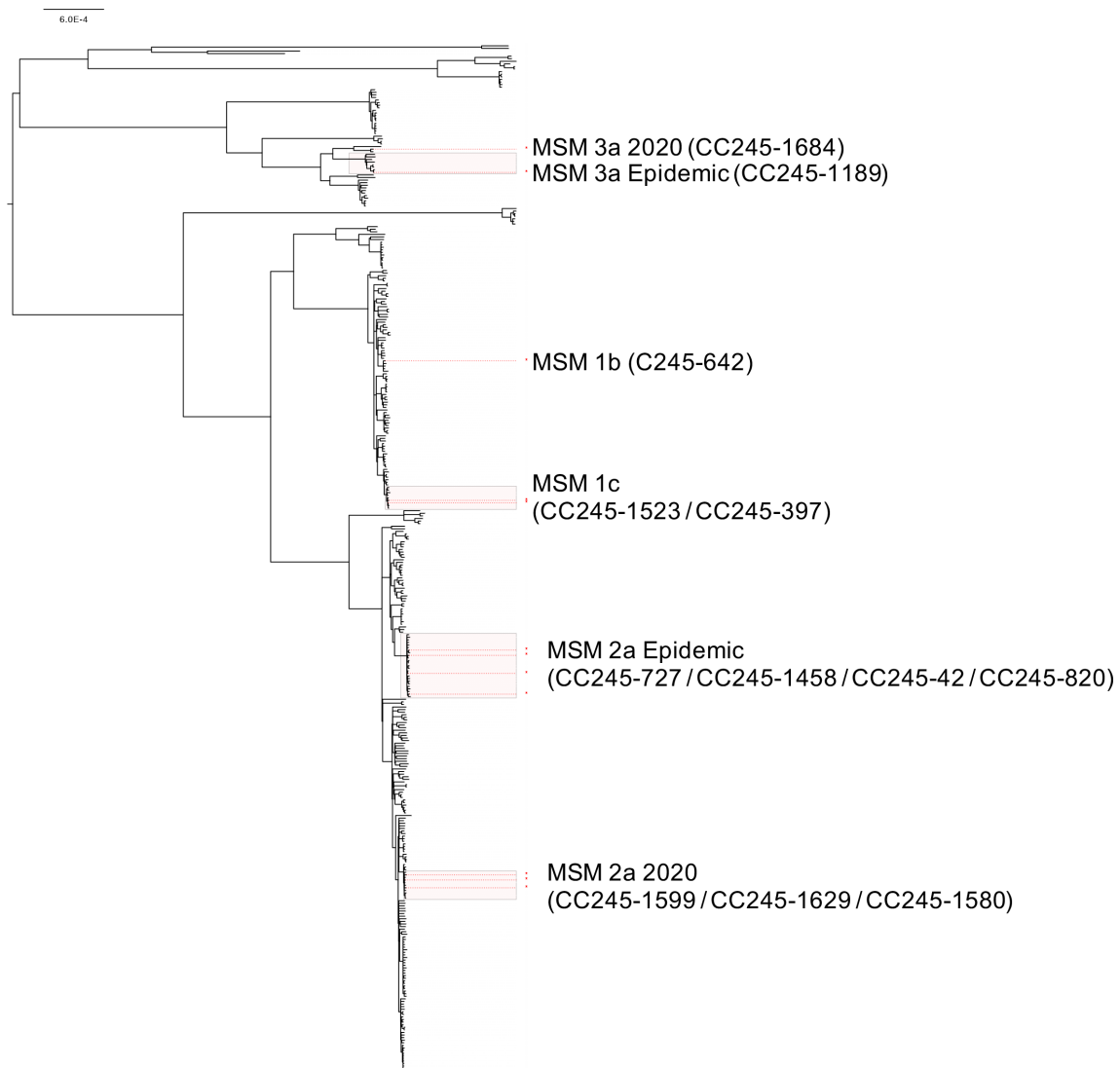


Fig. 4. Population structure of isolates of *S. flexneri* included in this study.

restrictions imposed in March 2020. Isolates belonging to the novel 2a MSM clade exhibited resistance to ciprofloxacin; although none of the isolates belonging to the 1b or 1c clades are resistant to azithromycin or ciprofloxacin, they are multidrug resistant (Table 1).

Since January 2019, prior to the implementation of lockdown restrictions in March 2020, transmission of *S. flexneri* among MSM had intensified. Despite advice to stay at home, and an initial decline in the number of cases in April 2020, the average number of diagnoses of *S. flexneri* in adult males in the second quarter of 2020 remained higher than during any quarter between mid-2015 and mid-2018. The different clades of *Shigella* contributing to the highest levels of transmission continue to fluctuate, although the drivers of the rise and fall of the dominant types remains unclear. Host immunity may be one factor influencing the change in dominant types observed over the last decade [16, 17].

Herd immunity may reduce circulation of specific serotypes, preventing epidemic strains from being sustained in the population, providing the opportunity for novel types, such as *S. flexneri* serotypes 1b and 1c described in this report, to find a niche. Waning antibody levels may result in the re-emergence of previous epidemic strains, as with the MSM 3a epidemic clone also described here.

Prior to 2019, sexual transmission in adult males was characterized by a single dominant type at any given time, with *S. flexneri* serotypes 3a, 2a and *S. sonnei* clades 2 and 5 emerging in successive waves between 2009 and 2018 [11, 13]. The emergence of each clade corresponded with the acquisition of the pK100 plasmid conferring resistance to azithromycin [12, 17, 18]. Since 2019, the three clades of *S. flexneri* serotype 2a, 1b and 1c, appear to be emerging concurrently, and all are susceptible to azithromycin. Furthermore, there is evidence that some isolates

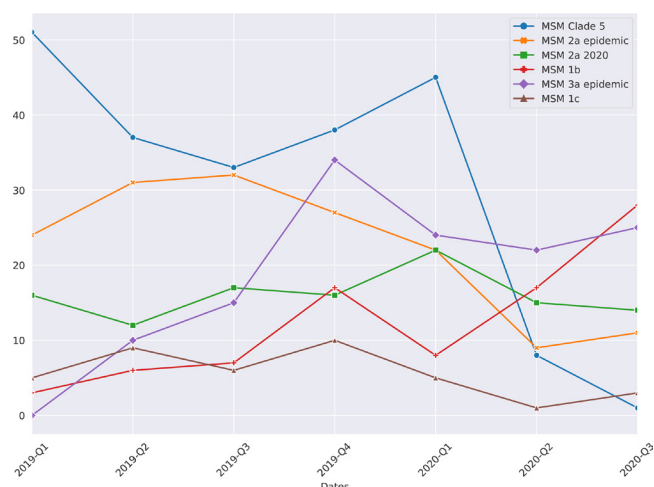


Fig. 5. Trends in cases of shigellosis-associated clusters with the six most common MSM-associated clusters.

within the re-emerging MSM 3a epidemic strain have lost pK100 and are no longer resistant to azithromycin. However, the emergence of a second MSM clade exhibiting ciprofloxacin resistance, the recommended first-line treatment for shigellosis, is a major public health concern [19].

The variation in AMR profiles observed in the different serotypes of *S. flexneri* circulating in the MSM community is a challenge for the clinical management of cases with severe symptoms where empirical treatment may be necessary. Resistance to azithromycin, and more recently to ciprofloxacin, has been observed in *S. flexneri* and *S. sonnei*, and outbreaks of extended-spectrum beta-lactamase-producing *S. sonnei* have been described [1, 11, 17–22]. Overuse of certain antimicrobials is likely to be a contributing factor in driving increasing resistance to the same class of antimicrobials. A combination of whole genome sequencing analysis, and improved collection of data on the antibiotics administered, clinical outcomes and exposure risks could contribute to the development of a predictive framework that will better inform guidelines on treatment and patient management and targeted public health messaging. Continued surveillance and monitoring to assess the impact of treatment regimens and interventions is essential.

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Author contributions

H.C., M.P. and T.D. analysed the data. T.D. and C.J. drafted the manuscript. H.C., M.P., K.S., C.J., G.G., T.D. and G.H. contributed to data interpretation and revised the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The authors declare that there is no requirement for ethical approval for this submission. This work was undertaken to inform the delivery of patient care and to prevent the spread of infection, defined as USUAL PRACTICE in public health and health protection.

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