Baker, Kate S; Dallman, Timothy J; Field, Nigel; Childs, Tristan; Mitchell, Holly; Day, Martin; Weill, François-Xavier; Lefèvre, Sophie; Tourdjman, Mathieu; Hughes, Gwenda; +2 more... Jenkins, Claire; Thomson, Nicholas; (2018) Genomic epidemiology of Shigella in the United Kingdom shows transmission of pathogen sublineages and determinants of antimicrobial resistance. Scientific reports, 8 (1). 7389-. ISSN 2045-2322 DOI: https://doi.org/10.1038/s41598-018-25764-3

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/4647681/

DOI: https://doi.org/10.1038/s41598-018-25764-3

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Genomic epidemiology of *Shigella* in the United Kingdom shows transmission of pathogen sublineages and determinants of antimicrobial resistance

Kate S. Baker, Timothy J. Dallman, Nigel Field, Tristan Childs, Holly Mitchell, Martin Day, François-Xavier Weill, Sophie Lefèvre, Mathieu Tourdjman, Gwenda Hughes, Claire Jenkins & Nicholas Thomson

*Shigella* are globally important diarrhoeal pathogens that are endemic in low-to-middle income nations and also occur in high income nations, typically in travellers or community-based risk-groups. *Shigella* phylogenetics reveals population structures that are more reliable than those built with traditional typing methods, and has identified sublineages associated with specific geographical regions or patient groups. Genomic analyses reveal temporal increases in *Shigella* antimicrobial resistance (AMR) gene content, which is frequently encoded on mobile genetic elements. Here, we whole genome sequenced representative subsamples of *S. flexneri* 2a and *S. sonnei* (n = 366) from the United Kingdom from 2008 to 2014, and analysed these alongside publicly available data to make qualitative insights on the genomic epidemiology of shigellosis and its AMR within the broader global context. Combined phylogenetic, epidemiological and genomic analyses revealed the presence of domestically-circulating sublineages in patient risk-groups and the importation of travel-related sublineages from both Africa and Asia, including ciprofloxacin-resistant sublineages of both species from Asia. Genomic analyses revealed common AMR determinants among travel-related and domestically-acquired isolates, and the evolution of mutations associated with reduced quinolone susceptibility in domestically-circulating sublineages. Collectively, this study provides unprecedented insights on the contribution and mobility of endemic and travel-imported sublineages and AMR determinants responsible for disease in a high-income nation.

*Shigella* are the most common bacterial cause of moderate to severe diarrhoea in children under 5 years old in Asia and Africa. In high income nations, shigellosis is often related to travel but can also circulate as a sexually transmissible illness of men who have sex with men (MSM). Among the four species comprising the genus *Shigella*, *S. sonnei* and *S. flexneri* cause the greatest disease burden. Among the diverse (>50) serotypes of *S. flexneri*, the 2a serotype is historically the most prevalent worldwide and in the United Kingdom (UK). However, increased resolution of *Shigella* species population structures has been provided by Whole Genome Sequence
Analysis (WGSA). *S. sonnei* is split into five WGSA subtypes called 'lineages', with the original four lineages being joined recently by a fifth reported in Latin America and Asia. *S. flexneri* has been similarly divided into seven WGSA subtypes called 'phyllogroups' (PGs). In addition to these species-defining studies, prevailing and emerging lower-order WGSA subtypes (usually called 'sublineages') of *S. sonnei* and *S. flexneri* have also been described in countries in Asia, the Middle East and Latin America. WGS has also been used to demonstrate the international movement of *Shigella* sublineages where WGSA subtypes prevailing in one geographical region are found in another, isolated from patients with an epidemiological link (e.g. travel). For example, a specific sublineage of *S. sonnei* that dominates in Israel circulates globally among Orthodox Jewish communities.

On top of this global population structure of shigelae WGSA subtypes, there is a horizontal and vertical transmission network of genes, including those encoding antimicrobial resistance (AMR). When WGSA subtyping is combined with epidemiological metadata and genome analyses (genomic epidemiology), the importance of AMR in shaping epidemic emergences and broader population structure in shigellae has been revealed. Studies over long time scales have shown the accumulation of AMR in *Shigella* over time, and both local and global epidemic emergences of WGSA subtypes have been found to be associated with resistance to therapeutically-important antimicrobial classes. AMR determinants in *Shigella* include Mobile Genetic Elements (MGEs), such as small and large resistance plasmids, and chromosomally-integrated islands, some of which have been found in multiple *Shigella* species. For example, the *Shigella* Resistance Locus Multiple Drug Resistance Element (SRL-MDRE) encoding resistance to ampicillin, chloramphenicol and tetracycline has been found in widely geographically-distributed *S. dysenteriae*, *S. flexneri* and *S. sonnei*. In addition to these horizontally-acquired AMR determinants, chromosomal mutations in the Quinolone Resistance Determining Region (QRDR) (that confer resistance to ciprofloxacin, the recommended treatment for shigellosis) have also been reported in shigellae isolated from Asia, Latin America, and high-income nations. *Ciprofloxacin*-resistant *Shigella* were recently recognised by the World Health Organisation as one of the top dozen agents for which new antimicrobials are urgently needed. Common AMR determinants being found in geographically-separated and genetically-distinct *Shigella* subtypes point to the independent mobility of AMR determinants as well as convergent evolution.

With an increased resolution and volume of information on the population structure of *Shigella* and its AMR determinants available, these data can be used to enhance surveillance in public health laboratories that are using whole genome sequencing, such as the Gastrointestinal Bacteria Reference Unit (GBRU) at Public Health England (PHE) in the UK. In the UK, shigellosis has historically been associated with travel and only low-levels of domestic acquisition (i.e. illness in patients without a recent history of travel to a shigellosis-endemic area). Recently however, epidemics of domestically-acquired shigellosis (*S. sonnei*, *S. flexneri* 2a and *S. flexneri* 3a) have been linked with transmission among adult males, and more specifically among men who have sex with men (MSM). As part of a recent study investigating AMR emergence among these parallel epidemics in MSM, we whole genome sequenced representative subsamples of *S. sonnei* (*n* = 187) and *S. flexneri* 2a (*n* = 179) from the UK sampled between 2008 and 2014. Here, in this allied study, we integrate this new cross sectional data from the UK with existing reference datasets of *Shigella* WGSA subtypes and AMR determinants from around the world to identify those responsible for shigellosis in the UK. By providing the context necessary for interpreting national surveillance patterns, we reveal important qualitative insights on the importation and domestic transmission of *Shigella* in the UK, as well as on the prevalence and mobility of AMR among these globally-important pathogens.

**Methods**

**Nationally representative isolates.** To investigate an epidemic phase of *Shigella* transmission associated with MSM, isolates of *S. sonnei* and *S. flexneri* 2a submitted to the GBRU at PHE were representatively subsampled, with a random sample of isolates from each year proportional to the total number of annual cases (for each species) being taken from across 2008–2014 (Fig. 1). In doing so, we aimed to balance an economically-practical number of isolates for WGSA, with the need to have sufficient statistical power to detect emerging sublineages of domestic transmission (this calculation was based on data in). For *S. flexneri* 2a, there were no selection criteria based on age, whereas for *S. sonnei* (owing to the larger number of cases that occur annually) selection was restricted to patients aged 16–60 years old. To focus on the epidemic domestic transmission, we sequenced a greater proportion of isolates from patients who did not report recent high-risk travel for both species (Fig. 1). The final proportions were as follows: for *S. sonnei* 3% (45 of 1539 cases) of travel-associated cases and 8% (142 of 1751 cases) of non-travel associated cases from patients aged 16–60 years old, and for *S. flexneri* 2a 9% (32 of 368 cases) of travel-associated cases and 18% (147 of 820 cases) of non-travel associated cases from patients of all ages. This resulted in a total of 187 *S. sonnei* and 179 *S. flexneri* 2a isolates (Fig. 1).

**Publicly available data.** Various *S. sonnei* reference isolates (*n* = 40) from other WGSA studies were included to provide interpretative context. Representative contextual isolates and the associated studies were as follows: the original four global lineages of *S. sonnei*, including those classified into the multidrug resistant and globally-disseminated ‘Global III’ sublineage; the newly identified global Lineage V and a regionally-epidemic Latin America sublineage, Latin America IIIa; a sublineage associated with transmission in Israel and Orthodox Jewish communities elsewhere; and a ciprofloxacin-resistant sublineage circulating in, and disseminating from, Asia, the ‘Central Asia III’ lineage (Supplementary Data). For *S. flexneri* 2a, isolates sequenced in a population-structure-defining study were included in analyses to anchor the positions of the *S. flexneri* 2a representative subsample, and investigate geographical associations. Specifically, this included representatives of all PGs (*n* = 9) and all PG3 *S. flexneri* 2a isolates (*n* = 69) (Supplementary Data).

**Bioinformatic analyses.** DNA was extracted and isolates whole genome sequenced as previously described. Whole genome sequence phylogenetic trees were constructed through mapping of sequence data
(using smalt, http://www.sanger.ac.uk/science/tools/smalt-0) to reference genomes (S. flexneri 2a 2457 T, S. sonnei 53 G), followed by removal of mobile (plasmids, insertion sequences, other described mobile islands) and repetitive elements (through removal of known repeat regions and repeat mapping discard), and sites of recombination (using gubbins)25, followed by extraction of variable sites (using snp_sites26). This resulted in multiple sequence alignments of 19348, 11141, and 9790 variant sites in length for S. flexneri (all PGs), S. flexneri (PG3 only), and S. sonnei respectively. Multiple sequence alignments were used to infer maximum likelihood phylogenetic trees using RAxML with 100 bootstrap replicates27. Acquired AMR genes were identified using ResFinder28 on draft genome assemblies (generated by)29 and QRDR mutations were detected as previously described7. All sequence data are publically available at the European Nucleotide Archive under project number PRJEB12097 (individual accessions in Supplementary Data).

Ethical statement. No individual patient consent was required or sought as PHE has authority to handle patient data for public health monitoring and infection control under section 251 of the UK National Health Service Act of 2006 (previously section 60 of the Health and Social Care Act of 2001). The project was reviewed and approved by the PHE Research Support and Governance Office and Caldicott Panel deemed to comply with public health surveillance standards.

Data availability statement. All sequencing and genome assembly data for isolates in this study are available under project number PRJEB12097 in the European Nucleotide Archive. Further detail linking isolates and sample accession numbers within the project are available in Supplementary Data.

Results

Mobility of S. sonnei and S. flexneri 2a WGSA subtypes. For both species, the whole genome phylogenies revealed a dominant subtype. Most S. flexneri 2a isolates (176 of 179) belonged to PG3 (Fig. 2A) and most (181 of 187) of the S. sonnei isolates belonged to Lineage III (Fig. 3). For S. flexneri 2a, this likely represents the strong association of the 2a serotype with the PG3 WGSA subtype. The association was not exclusive though as one PG2 and two PG1 isolates were among the S. flexneri 2a samples, reaffirming that serotype switching between PGs occurs8. For S. sonnei, the dominance of Lineage III reflects a greater burden of disease caused by this lineage, as previously shown6. Despite the Lineage III dominance however, further diversity of S. sonnei was detected in the UK samples. Specifically, we observed two Lineage V isolates from domestically-acquired cases (despite Lineage V only having been previously reported in Latin America or Africa)7, one Lineage I isolate from a patient recently-returned from Asia and three Lineage II isolates from patients without a history of recent travel (Fig. 3). In fact, the only lineage of S. sonnei not detected, Lineage IV, has yet to be seen outside of its original description6.

In addition to this diversity at the highest level of nomenclature (i.e. lineage/PG), analysis at the sub-lineage level enabled further examination of the role of travel in the epidemiology of shigellosis in the UK. Travel-associated isolates were often phylogenetically proximate to reference isolates from that same region, differing by as few as 3 SNPs (Figs 2B and 3). For S. flexneri PG3, there was a sublineage related to travel from Asia,
containing Asian reference isolates as well as most (11 of the 17) of the patients recently returned from Asia (specific locations were India, Pakistan, Sri Lanka and Afghanistan). To reflect these origins, and mirror *S. sonnei* nomenclature, this sublineage was designated *S. sonnei* Central Asia III (recently described as disseminating from Asia 14) was similarly detectable (Fig. 3). Specifically, 12 of the 15 isolates of *S. sonnei* from patients who had recently travelled to Asia (China and India) belonged to this sublineage. In addition to these Asian-travel related sublineages, there was a similar *S. sonnei* sublineage related to travel from Africa (see African travel related, Fig. 3). Specifically, the majority (13 of 19) of isolates from patients who had recently travelled to Africa belonged to a sublineage within the Global III sublineage (Fig. 3). Twelve of these 13 patients had recently returned from Egypt, and one from Nigeria, so it is likely that this sublineage represents the repeated importation of a sublineage that is nationally- or regionally-dominant in Northern Africa. In addition to these Asian- and African-related travel lineages, an *S. sonnei* isolate belonging to the Latin America IIIa sublineage was detected in a patient who had recently returned from a Latin American country where this lineage is highly prevalent7.

Figure 2. *Shigella flexneri* 2a UK cross section in context. (A) The smaller tree shows the broad context of phylogroups (PG) 1–7 of *S. flexneri* and intercontinental MSM-associated (MSMA) *S. flexneri* 3a, with the relationships of three non-PG3 isolates from this study. PG3 is overlaid by a grey polygon in the smaller tree and is shown in full in the larger tree. (B) The larger tree is a mid-point rooted maximum likelihood tree and shows the detailed evolutionary relationships of UK cross section isolates (black branches) with reference isolates (coloured branches). Reference isolate branches are coloured by region of sample origin, and region of recent travel is overlaid for UK samples as coloured circles on tree tips (both coloured according to the inlaid region key). Two MSM-associated sublineages are highlighted by grey boxes, and the *S. flexneri* 2a Central Asia lineage is indicated at the defining internal node. AMR features are shown in adjacent tracks. The leftmost track shows the presence of QRDR mutations and one ‘Other’, which designates a quadruple mutant also carrying the qnrS1 gene. Subsequent gray scale tracks indicate the presence of antimicrobial resistance genes (ARGs, labelled above) associated with known *Shigella* MGEs. Specifically (from left to right), the Tn7/Int2, SRL-MDRE, Small R-plasmids, Minor clade plasmid, pKSR100 (Table 1).
Sublineages transmitting domestically in patient communities were also detectable among the isolates. A separate analysis of this data incorporating patient age and gender identified the six sublineages associated with transmission in MSM (MSM-associated, Figs 2 and 3). For *S. flexneri* 2a there was one large (47 isolates), low-diversity (maximum pair-wise distance 20 SNPs) MSM-associated sublineage, and one smaller (7 isolates), higher-diversity (maximum pair-wise distance 148 SNPs) MSM-associated sublineage (Fig. 2). Among the *S. sonnei* isolates, there were four MSM-associated sublineages with maximum pairwise distances of between 15 and 30 SNPs (Fig. 3). One MSM-associated sublineage of *S. sonnei* was nested within the Central Asia III sublineage, and three other MSM-associated *S. sonnei* sublineages belonged to the Global III sublineage (Fig. 3), which is thought to have disseminated globally after the acquisition of the Tn7/Int2 AMR determinant. In addition to the MSM-associated sublineages, isolates belonging to the Orthodox Jewish community-associated sublineage (also within Global III sublineage) were detected from years spanning 2008–2014 (Fig. 3). This is consistent with a previous study that demonstrates that this Orthodox Jewish community-associated sublineage is circulating between Israel and the UK, where it causes domestically-transmitted outbreaks.

---

**Figure 3.** *Shigella sonnei* UK cross section in context. The midpoint-rooted maximum likelihood phylogenetic tree shows isolates from this study in the context of five global lineages of *S. sonnei*. Lineage III has further relevant sublineages indicated at the internal nodes and MSM-associated sublineages are highlighted in grey. Isolates from patients recently returned from endemic regions are indicated by circles overlying the tree tips which are coloured according to the inlaid key. Reference isolates from previously described WGS subtypes (lineages/sublineages) are indicated in the leftmost adjacent track, and tree branches for references isolates are also coloured according to the inlaid key. AMR features are shown in subsequent tracks. The second leftmost track shows the presence of QRDR mutations and two isolates containing ‘Other’ quinolone resistance determinants (including an isolate with a single QRDR mutation and a *qepA* gene, and an isolate containing a *qnrB19* gene). Subsequent gray scale tracks indicate the presence of antimicrobial resistance genes (ARGs, labelled above) associated with known *Shigella* MGEs. Specifically (from left to right), the Tn7/Int2, SpA, MSM-associated sublineage 3 plasmid, pKSR100, SRL-MDRE (Table 1).
alternatively could result from misreporting of travel histories). In our study, domestically-acquired cases of present in the travel-related sublineages and likely represent onward transmission from travel-related cases (or Asia sublineages. As our study focused on domestically-acquired shigellosis, we were uniquely able to follow ing, particularly when they are resistant to key antimicrobial classes such as the ciprofloxacin-resistant Central spectrum beta lactamases and plasmid-encoded quinolone resistance genes were rare (10 and 3 of 366 isolates (ARGs) encoded resistance against virtually all known antimicrobial classes. However, genes encoding extended fully resistant mutants 14, and might indicate the potential for ciprofloxacin resistance to evolve in sublineages D87Y mutation (Fig. 3). These single mutations have previously shown to be step-wise toward the evolution of ing, particularly when they are resistant to key antimicrobial classes such as the ciprofloxacin-resistant Central

Fig. 3). In addition to these triple-mutant sublineages that evolved in Asia and spread to the UK, isolates and sub-

transmitting locally in the UK.

This study reveals important information about the epidemiology of shigellosis in a high-income nation where shigellosis is frequently related to travel. Indeed, this study confirms that importation of sublineages from both Africa and Asia for S. sonnei, and a similar Asian-travel related sublineage of S. flexneri 2a (Fig. 2). Other closely-related small resistance plasmids, specifically the S. sonnei SpA plasmid, reported in the first sequenced genome32, and the resistance plasmid pSFxv_3, originally described in the Chinese epidemic S. flexneri Xv33, were also near-ubiquitous among the isolates (Figs 2 and 3, Table 1). Large resistance plasmids encoding azithromycin, and other, antimicrobial resistances were also found in isolates from MSM-associated sublineages (described more fully elsewhere)33.

Owing to its importance for treatment, ciprofloxacin resistance was also examined among the isolates. Remarkably, for both Shigella species, triple QRDR mutations conferring resistance to ciprofloxacin were specifically-associated with the Central Asia sublineages (S. flexneri 2a Central Asia in Fig. 2, Central Asia III in Fig. 3). In addition to these triple-mutant sublineages that evolved in Asia and spread to the UK, isolates and sub-

Table 1. Antimicrobial resistance determinants of Shigella and species distributions. The strA gene has been interrupted by the introduction of dfra14 on this plasmid meaning no Streptomycin resistance would be conferred.

| Type                      | Name                        | Resistance genes     | Antimicrobial classes          | Species reports |
|---------------------------|-----------------------------|----------------------|-------------------------------|----------------|
| Chromosomal Islands       | SRL-MDRE                    | tetB, bblaECA_1, catA1 | Tetracyclines, Ampicillin, Chloramphenicol | S. dysenteriae 13 |
|                           | Tn7/Int2                    | aadA1, dfrA1         | Aminoglycosides, Trimethoprim | S. flexneri 2a (this study) |
|                           | pSS046_spA                  | sul2, strA/strB, tetA | Sulfonamides, Streptomycin, Tetracyclines | S. sonnei 34,35 |
| Small resistance plasmids | pABC-3                      | dfrA14, sul2, strB    | Trimethoprim, Sulfonamides    | S. sonnei 34,35 |
|                           | psFsv_3                     | strA/strB, sul2      | Streptomycin, Sulfonamides    | S. flexneri Xv33, S. flexneri 2a (this study) |
| Large azithromycin        | pKSR100                     | mphA, ermB, bblaT33_13, ada15, dfrA17, sul1 | Macrolides, Macrolides, Ampicillin, Aminoglycosides, Trimethoprim, Sulfonamides | MSM-associated: S. flexneri 3a13, S. flexneri 2a13, S. sonnei13 |
| resistance plasmids       | Minor MSMA sublineage plasmid | mphA, tetA, bblaT33_1a | Macrolides, Tetracyclines, Ampicillin | S. flexneri 2a13 |
|                           | MSMA sublineage 3 plasmid   | mphA, dfrA1, sul1   | Macrolides, Trimethoprim, Sulfonamides | S. sonnei 23 |

Shared AMR determinants among Shigella species. There were various horizontally-transmissible AMR genes among the isolates, often carried on known MGEs (Table 1). These Antimicrobial Resistance Genes (ARGs) encoded resistance against virtually all known antimicrobial classes. However, genes encoding extended spectrum beta lactamases and plasmid-encoded quinolone resistance genes were rare (10 and 3 of 366 isolates respectively. Supplementary Data). There was a chromosomally-encoded MGE encoding multiple-drug resistance in both S. sonnei and S. flexneri (i.e. the Tn7/Int2 determinant and the SRL-MDRE respectively, Table 1, Figs 2 and 3). Notably, the SRL-MDRE was also present in the only S. sonnei isolate belonging to the Latin America IIIa sublineage (Fig. 3), congruous with the known AMR profile of this sublineage in its endemic region2. This isolate also contained the small resistance plasmid pABC-3, typical of Latin America IIIa S. sonnei, and a similar Asian-travel related sublineage of S. flexneri (Fig. 2). Other closely-related small resistance plasmids, specifically the S. sonnei SpA plasmid, reported in the first sequenced genome32, and the resistance plasmid pSFxv_3, originally described in the Chinese epidemic S. flexneri Xv33, were also near-ubiquitous among the isolates (Figs 2 and 3, Table 1). Large resistance plasmids encoding azithromycin, and other, antimicrobial resistances were also found in isolates from MSM-associated sublineages (described more fully elsewhere)33.

Discussion
This study reveals important information about the epidemiology of shigellosis in a high-income nation where shigellosis is frequently related to travel. Indeed, this study confirms that importation of Shigella sublineages through travel is a driver of shigellosis in the UK. The most striking examples of this are the repeated importation of sublineages from both Africa and Asia for S. sonnei, and a similar Asian-travel related sublineage of S. flexneri 2a. These sublineages likely represent epidemiologically prevailing sublineages circulating in endemic regions that disseminate through travel, as previously shown for the Central Asia III sublineage of S. sonnei14 and for African and Asian-travel related sublineages of S. flexneri 3a15. The importation of sublineages is concern-

including in one of the domestically-transmitting MSM-associated S. sonnei sublineages which contained a gyrA D87Y mutation (Fig. 3). These single mutations have previously shown to be step-wise toward the evolution of fully resistant mutants14, and might indicate the potential for ciprofloxacin resistance to evolve in sublineages transmitting locally in the UK.
travel-related sublineages often appeared to be dead-end transmissions (i.e. did not form larger clusters of isolates from domestically-acquired infections). This is consistent with recent comprehensive WGSA surveillance of S. sonnei in the UK where travel-related cases were typically in genetic clusters of only small numbers of isolates

In this study however, we did observe one antimicrobial resistant sublineage that was imported and then went on to contribute to a domestically-transmitting epidemic. Specifically, an MSM-associated sublineage of S. sonnei was nested within the Central Asia III sublineage, indicating that a newly imported AMR strain can cause domestically-propagated epidemics they should enter an appropriate transmission network

Unfortunately, biases in our subsample (proportional sampling at different depths for travel and non-travel related isolates, as well as age-restrictions for S. sonnei) make it inappropriate for us to draw quantitative inferences on the relative contribution of this importation to the shigellosis burden in the UK. However, recent results from routine whole genome sequencing surveillance of S. sonnei in the UK for the period August 2015–January 2016 show that approximately 60% of genetic clusters are likely related to travel

In addition to directly causing domestically-transmitting epidemics, imported strains can bring in new AMR determinants that might be transferred into locally-circulating sublineages. Encouragingly, there was little evidence for the mobility of mutated QRDRs from imported lineages through chromosomal recombination. However, this was not the case for ARGs carried on MGEs where there was evidence for the exchange of AMR determinants within the UK. For example, the small resistance plasmid carried in the imported Latin American IIIa S. sonnei (pABC-3) was also found in the domestically-transmitting MSM-associated sublineages of S. flexneri 2a (Fig. 2) and S. flexneri 3a11 (Table 1). This indicates the potential for imported AMR determinants to horizontally transmit to locally-circulating sublineages and contribute to epidemic emergences. The imported Latin American IIIa S. sonnei isolate also carried the SRL-MDR, which is rare in S. sonnei outside of Latin America, but could mobilise to other pathogens capable of genetic exchange of AMR determinants between imported and locally-circulating strains means that the public health impact of the importation of AMR pathogens may not be the disease caused by that specific pathogen, but rather the mobilisation of the AMR determinants into other already locally-adapted pathogens and/or patient microbiota, which would then act as a reservoir for AMR emergence. In this way, imported AMR pathogen enrich the UK AMR pool, and may only be noticed only when antimicrobial usage is sufficient to drive their spread to reportable pathogens. Indeed, the ability for horizontal transfer of an AMR plasmid to drive new population-level epidemics is shown in our allied study focused on the MSM-associated sublineages.

The mobility of AMR determinants among Shigella sp. is more broadly evidenced by the detection of the same AMR determinants among the diverse global data available. Analysis of previous reports and data from this study show that different Shigella species across disparate geographical locations share common AMR MGEs (Table 1). That this mere handful of AMR determinants dominate the genetic landscape of Shigella is remarkable considering the breadth of what shigellae (globally ubiquitous, genetically-diverse pathogens capable of lateral gene transfer) would have the opportunity to acquire in the global enteric milieu. The presence of epidemiologically-dominant sublineages with stable AMR determinant associations is commonly interpreted to result from epidemiological success of the sublineage consequent to the conferred AMR phenotype(s). However, in addition to that effect, the small number of recurrent AMR determinants among enormously diverse shigellae might suggest that the success of such AMR determinant-sublineage combinations may also be attributable to some measure of fitness-matching of the AMR determinant for that sublineage/species. It is picking apart these finer details of what limits and drives AMR flow among pathogenic organisms such as Shigella that will allow us to understand and perhaps eventually predict new emergences to prevent their occurrence.

References

1. Liu, J. et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet 388, 1291–1301, https://doi.org/10.1016/S0140-6736(16)31529-X (2016).
2. Kotloff, K. L. et al. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bulletin of the World Health Organization 77, 651–666 (1999).
3. Dritz, S. K. & Back, A. F. Letter: Shigella enteritis venerally transmitted. The New England journal of medicine 291, 1194 (1974).
4. Livio, S. et al. Shigella isolates from the global enteric multicenter study inform vaccine development. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 59, 923–946, https://doi.org/10.1093/cid/ciu408 (2014).
5. Borg, M. L. et al. Ongoing outbreak of Shigella flexneri serotype 3a in men who have sex with men in England and Wales, data from 2009–2011. Euro surveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin 17 (2012).
6. Holt, K. E. et al. Shigella sonnei genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. Nature genetics 44, 1056–1059, https://doi.org/10.1038/ng.2369 (2012).
7. Baker, K. S. et al. Whole genome sequencing of Shigella sonnei through PulseNet Latin America and Caribbean: advancing global surveillance of foodborne illnesses. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, https://doi.org/10.1111/cmi.13735 (2017).
8. Connor, T. R. et al. Species-wide whole genome sequencing reveals historical global spread and recent local persistence in Shigella flexneri. Elife 4, e07335, https://doi.org/10.7554/elif.e07335 (2015).
9. Holt, K. E. et al. Tracking the establishment of local endemic populations of an emergent enteric pathogen. Proceedings of the National Academy of Sciences of the United States of America 110, 17522–17527, https://doi.org/10.1073/pnas.1308632110 (2013).
10. Baker, K. S. et al. Travel- and Community-Based Transmission of Multidrug-Resistant S. sonnei Lineage among International Orthodox Jewish Communities. Emerging infectious diseases 22, 1545–1553, https://doi.org/10.3201/eid2209.151953 (2016).
11. Chung The, H. et al. Introduction and establishment of fluoroquinolone-resistant Shigella sonnei into Bhutan. Microb Genom 1, e000042, https://doi.org/10.1099/mgen.0.000042 (2015).
12. Zhang, N. et al. Genomic Portrait of the Evolution and Epidemic Spread of a Recently Emerged Multidrug-Resistant Shigella flexneri Clone in China. Journal of clinical microbiology 52, 1119–1126, https://doi.org/10.1128/JCM.02699-13 (2014).
13. Baker, K. S. et al. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. The Lancet infectious diseases 15, 913–921, https://doi.org/10.1016/S1473-3099(15)00002-X (2015).
14. Chung The, H. et al. South Asia as a Reservoir for the Global Spread of Ciprofloxacin-Resistant Shigella sonnei: A Cross-Sectional Study. PLoS medicine 13, e1002055, https://doi.org/10.1371/journal.pmed.1002055 (2016).
Acknowledgements
The authors are grateful to the administrative, sequencing and pathogen informatics teams at the Wellcome Trust Sanger Institute and helpful scientists who commented on the work in progress during formal and informal presentations. The authors also thank John Were for his contribution to managing epidemiological data. This work was supported by Wellcome Trust grant number 206194, and KSB is supported by a Wellcome Trust Clinical Research Career Development Fellowship (106690/A/14/Z).

Author Contributions
K.S.B., T.D., N.F., G.H., C.J., N.R.T. conceived and developed study. K.S.B. directed research, performed analyses and wrote the manuscript drafts. T.D., N.F., C.J., N.R.T. subsampled prepared English isolates for sequencing. F.X.W. and S.L. selected, susceptibility tested and prepared French isolates for sequencing. N.F., H.M., G.H. identified UK patients with repeat infections. M.T. conducted epidemiological interviews of French patients. M.D. conducted susceptibility testing of English isolates. All authors contributed to and approved the final manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-25764-3.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license, you may need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018