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**Title**

Intercontinental dissemination of azithromycin resistant shigellosis through sexual transmission: a cross sectional study

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## ABSTRACT

**Background:** Shigellosis is an acute, severe bacterial colitis that, in high-income countries, is typically associated with travel to high-risk regions (Africa, Asia, and Latin America). Since the 1970s, shigellosis has also been reported as a sexually transmitted infection in men who have sex with men (MSM), in whom transmission is an important component of shigellosis epidemiology in high-income nations. We aimed to use sophisticated subtyping and international sampling to determine factors driving shigellosis emergence in MSM linked to an outbreak in the UK.

**Methods:** We did a large-scale, cross-sectional genomic epidemiological study of shigellosis cases collected from 29 countries between December, 1995, and June 8, 2014. Focusing on an ongoing epidemic in the UK, we collected and whole-genome sequenced clinical isolates of *Shigella flexneri* serotype 3a from high-risk and low-risk regions, including cases associated with travel and sex between men. We examined relationships between geographical, demographic, and clinical patient data with the isolate antimicrobial susceptibility, genetic data, and inferred evolutionary relationships.

**Findings:** We obtained 331 clinical isolates of *S flexneri* serotype 3a, including 275 from low-risk regions (44 from individuals who travelled to high-risk regions), 52 from high-risk regions, and four outgroup samples (ie, closely related, but genetically distinct isolates used to determine the root of the phylogenetic tree). We identified a recently emerged lineage of *S flexneri* 3a that has spread intercontinentally in less than 20 years throughout regions traditionally at low risk for shigellosis via sexual transmission in MSM. The lineage had acquired multiple antimicrobial resistance determinants, and prevailing sublineages were strongly associated with resistance to the macrolide azithromycin. Eight (4%) of 206 isolates from the MSM-associated lineage were obtained from patients who had previously provided an isolate; these serial isolations indicated atypical infection patterns (eg, reinfection).

**Interpretation:** We identified transmission-facilitating behaviours and atypical course(s) of infection as precipitating factors in shigellosis-affected MSM. The intercontinental spread of

antimicrobial-resistant shigella through established transmission routes emphasises the need for new approaches to tackle the public health challenge of sexually transmitted infections in MSM.

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## INTRODUCTION

*Shigella sp.* are Gram negative bacteria that cause shigellosis, a faeco-orally transmitted disease characterised by severe colitis, induced by a very low infectious dose. In low-income countries, shigellae are one of the top four attributable causes of moderate-severe diarrhoea in children under five years old, an illness that kills approximately 750,000 per annum.<sup>1, 2</sup> In these areas, *S. flexneri* and *S. sonnei* are the main causative agents of endemic shigellosis, causing 53 and 32% of the burden respectively.<sup>3</sup> Contrastingly, in high-income countries *S. sonnei* causes sporadic disease often unlinked with travel, whereas cases of *S. flexneri* shigellosis are typically associated with recent travel to regions that are ‘high risk’ for contracting diarrhoeal disease, previously defined as, Asia, Africa and Latin America.<sup>4</sup>

In addition to this global epidemiology as a diarrhoeal pathogen, shigellosis can also be a sexually transmitted infection (STI) in men-who-have-sex-with-men (MSM) in whom, oro-anal contact and coinfection with HIV are key risk factors (between 37 and 75% of MSM shigellosis cases are HIV-positive).<sup>5-9</sup> The first report of MSM-associated shigellosis in “San Francisco’s gay community” was reported in 1974,<sup>10</sup> and the proportion of shigellosis in the city attributed to adult males quickly rose to 65 – 70%.<sup>7</sup> This increased role of adult males was mirrored nationally,<sup>11</sup> and a subsequent case-control study demonstrated that being MSM was the largest population attributable fraction (0.73) risk factor for shigellosis in adult males.<sup>5</sup> As well as continued reports in Northern America,<sup>6-8, 11, 12</sup> MSM-associated shigellosis outbreaks have also been reported in Oceania, Asia and Europe.<sup>4, 13-15</sup> Despite its importance to the epidemiology of shigellosis in high income countries, outbreaks of MSM-associated

shigellosis are frequently investigated with only basic microbiological subtyping and as small-scale epidemics in geographically restricted areas, with no investigation into possible connectivity with contemporaneous outbreaks in other countries.

In 2009 an outbreak of MSM-associated shigellosis caused by *S. flexneri* serotype 3a was reported in the United Kingdom (UK). The outbreak was identified by an increase in *S. flexneri* shigellosis in middle-aged males who did not have a history of travel to high-risk regions.<sup>4</sup> The association of this outbreak with MSM was confirmed by semi-structured interviews covering sexual and other risk behaviours, which confirmed that the majority of cases (78%, 21 of 27) were sexually active MSM who commonly reported high numbers of sexual partners, oro-anal contact, rare condom use, and co- or recent infection with other STIs.<sup>9, 16</sup> The outbreak serotype (3a) has also been reported as having replaced *S. sonnei* in Canadian MSM outbreaks, and has been identified in MSM-associated shigellosis in Australia.<sup>4, 12, 15</sup> In the UK, the outbreak serotype is now considered endemic and is responsible for the majority of *flexneri* shigellosis nationwide, having replaced *S. flexneri* 2a as the most common serotype (though is still largely restricted to MSM).<sup>4</sup>

To further investigate this growing public health concern, we collected over 300 clinical isolates of *S. flexneri* 3a. We combined traditional epidemiological approaches with whole-genome-sequence subtyping and characterisation of the isolates to reveal novel insights into the emergence and epidemiology of shigellosis in MSM. To identify factors driving shigellosis emergence in MSM and distinguish MSM-associated cases from those acquired through travel, we intensively sampled *S. flexneri* 3a from the United Kingdom. Samples from MSM-associated outbreaks in other countries, as well as samples from nations endemic for shigellosis were also included to determine the international context for shigellosis emergence in MSM and query the connection between international outbreaks.

## **METHODS**

### **Study design and isolate collection**

In this cross-sectional study, we representatively sampled all (both travel and domestically acquired) isolates of *S flexneri* serotype 3a isolated in the UK in 2004–13, including during an outbreak that began in 2009. To contextualise this outbreak, we included smaller (although still representative) numbers of isolates from the French reference laboratory and three high-risk areas (Africa, Asia, and Latin America). To determine whether the outbreak was connected to other international outbreaks in MSM, we also included isolates from MSM-associated outbreaks in Canada and Australia. Correlating the genomic analyses of these isolates with clinical data from patients provided novel insights into the infection and transmission of antimicrobial resistance and diarrhoeal pathogens in MSM.

For low-risk regions, we obtained representative *S flexneri* 3a isolate samples submitted to the Gastrointestinal Bacteria Reference Unit, Public Health England in 2004–13 (UK; appendix) and the French National Reference Center for *Escherichia coli*, *Shigella*, and *Salmonella* at the Pasteur Institute (France; appendix), or selected isolates from the Public Health Laboratory Toronto, Public Health Ontario (Canada) from male patients who self-reported oro-anal contact as a risk factor for shigellosis during public health follow-up interviews and the State Enteric Reference Laboratories in Victoria and New South Wales (Australia). For high-risk regions, we obtained isolates chosen by reference laboratories as representative of the *S flexneri* diversity present in those regions for a species-wide study (Connor TR, unpublished) as well as isolates from French Guiana in Latin America (also chosen by reference laboratories as representative). These isolates were complemented by isolates from patients in low-risk regions whose disease was contracted through recent travel to high-risk regions (appendix). Patients in low-risk regions were defined as having either a suspect travel history (recent travel to a high-risk region) or a non-suspect travel history (either no recent travel, unknown travel history, or recent travel to low-risk regions). We



obtained four isolates, which were known to be phylogenetically remote from other *S flexneri* serotype 3a (Connor TR, unpublished), for use in phylogenetic outgrouping (ie, to determine the root of the phylogenetic tree).

We analysed traditional epidemiological variables, including date and patient demography (location, sex, age, and travel history) alongside isolate variables (including phylogenetic associations and features of antimicrobial resistance).

We did susceptibility testing against ampicillin, streptomycin, tetracycline, trimethoprim, sulfonamide, nalidixic acid, ciprofloxacin, cefotaxime, and ertapenem with Isosensitest (Oxoid; Basingstoke, UK), Muller-Hinton agar (Oxoid), or the Etest macromethod (bioMérieux; Crappone, France). This last test was used to determine the minimum inhibitory concentrations against azithromycin (appendix).

### **Sequencing data and quality control**

Sequencing data was generated by study authors from genomic DNA at three collaborating sites for this study with Illumina platforms (San Diego, CA, USA; appendix). All sequence data is available at the European Nucleotide Archive (appendix). Sequencing data was de novo assembled and verified by in-silico multilocus sequence typing, as previously described.<sup>17</sup> Phred (>50) and mapping (>30) quality filters were applied in downstream processing. The annotated contiguous sequence of the multidrug resistance plasmid (pKSR100) from sample SF7955 is available at the European Nucleotide Archive under accession number LN624486.

### **Bioinformatic analyses**

To construct the phylogenetic tree of the full collection, we mapped isolate sequence data against *S flexneri* serotype 2a strain 301 (NCBI accession number NC\_004337.2), generated pseudosequences incorporating single-nucleotide polymorphisms (SNPs), and removed

mobile elements and pathogenicity islands as previously described.<sup>17</sup> The resulting alignment was stripped of suspected sites of recombination, and we used the remaining 19 940 SNP sites to construct a phylogenetic tree using RAxML version 7.8.6 as previously described.<sup>17</sup> We constructed the pKSR100 phylogeny similarly from plasmid-containing ( $\geq 96\%$  coverage) isolates and compared it with the genome tree. The multiple sequence alignment for the dated phylogeny of the MSM-outbreak associated lineage isolates was constructed similarly, mapping against a representative draft genome assembly (sample ERS369461). This generated a final alignment with 1243 SNPs, which underwent Bayesian evolutionary analysis by sampling trees (BEAST)<sup>18</sup> with a strict molecular clock and both constant and exponential population growth models (congruous results), accounting for invariant sites. In all cases, isolate sequencing data covered at least 96% of the reference.

For genetic analyses, we annotated isolate assemblies and detected antimicrobial resistance genes (ARGs) as previously described.<sup>17</sup> Further ARGs were identified by BLAST, and non-synonymous changes in GyrA and ParC protein sequences, and integron insertion into plasmid pKSR100 were identified manually.

### **Role of the funding source**

The funding source had no role in study design, data collection, analysis, and interpretation, writing the manuscript or decision to submit. The corresponding author had full access to all the data, and was responsible for the decision to submit the manuscript.

## **RESULTS**

Here we representatively sampled all (both travel and domestically acquired) isolates of *Shigella flexneri* serotype 3a isolated in the United Kingdom while a novel sexually transmitted lineage emerged and became endemic. To contextualise the outbreak, we included smaller (though still representative) numbers of isolates from the French reference laboratory and high-risk regions. To determine whether the outbreak was connected to other

international MSM-outbreaks, we also included isolates from MSM-associated outbreaks in Canada and Australia. Correlating the genomic analyses of these isolates with clinical data from patients provided previously unachievable insights into the infection and transmission of antimicrobial resistance and diarrhoeal pathogens in MSM.

### **The emergence of a new lineage of *Shigella flexneri***

To determine the global context of the *S. flexneri* 3a isolates from the United Kingdom, a phylogeny of the diverse *S. flexneri* 3a collection was constructed and related with patient gender and age, geographical information regarding the location of isolation, and patient travel history (Figure 1). This led to the identification of three main phylogenetic lineages (Figure 1A).

Two of these lineages were related to high-risk regions, with isolates collected from patients in high-risk regions clustering with isolates collected from patients living in low-risk regions, who had suspect travel histories to those high-risk regions. This resulted in the designation of an Asia-associated lineage comprised primarily (75%, 38 of 52 isolates) of isolates from patients in Asia and patients from low-risk regions who had recently travelled to Asia (Figure 1A,B), and an Africa-associated lineage that was similarly comprised (75%, 51 of 69 isolates, Figure 1A,B). Both lineages contained isolates from patients with similar age profiles; being distributed across the very young, middle aged and very old age groups (Figure 1C).

Finally, there was a monophyletic lineage almost exclusively (97% 199 of 206 isolates) comprised of isolates from patients living in low-risk regions with non-suspect travel histories (no recent travel to high-risk regions), with the exception of the seven isolates associated with Central/South America (Figure 1A,B). Distinct from the two lineages related to high-risk regions, the majority (97%, 199 of 205) of the isolates in this lineage were obtained from male patients (Figure 1B), with almost no patients being either very-young or very-old (Figure 1C). This was denoted the MSM-outbreak associated lineage (see next section). To further investigate the emergence and geographic distribution of this lineage, a lineage-specific, dated phylogeny was constructed (Figure 2). This revealed that the lineage emerged

(shared a most-recent-common-ancestor) between the years 1996 and 1998 (Figure 2). Within the lineage, isolates from patients in individual low-risk region countries did not cluster together phylogenetically and were found in multiple positions throughout the phylogeny, interspersed with isolates from other countries. This indicated that evolutionary relationships were not primarily determined by geographical origin (with the exception of isolates from French Guiana which clustered together). Collectively, this demonstrated that a recently emerged monophyletic lineage of *S. flexneri* 3a was circulating intercontinentally across regions traditionally considered low-risk for shigellosis.

### **Epidemiological association and infection patterns in MSM**

The lineage was named the MSM-outbreak associated lineage, as it was strongly associated with MSM. This link was suggested by the gender, age and travel history biases (above) and confirmed by the association of lineage with patient sexuality. In total, sexuality data was available for 46 patients in this study including 41 from the United Kingdom (obtained through epidemiological interview of 19% (40 of 207) of all UK patients revealing 36 MSM and 5 heterosexuals) and 5 from Canada (male patients self-reporting oro-anal contact, [Table S1, Appendix \[B\]](#)). Overall, 40 of 41 MSM patients were infected with isolates clustering within the MSM-outbreak associated lineage, and only 1 of 5 heterosexual patients was infected with isolates from this lineage (the others were in the Asia- and Africa-associated lineages). This demonstrated that patient sexuality was strongly associated ( $p=0.00002$ , Fisher's exact test) with lineage. Thus, the MSM-outbreak associated lineage was linked to MSM through both epidemiological evidence and biases in patient demography.

Patient clinical data also demonstrated two distinct resampling patterns among paired isolates ( $n=8$ ) from the same patient. Isolates that were collected within three months of each other (9 - 95 days apart) were neighbored phylogenetically, and separated by few (0 - 4) SNPs (Patient pairs, Figure 2). However, isolates from the same patient that were separated by five months or more (154 - 911 days) showed greater phylogenetic distinction (18 - 128 SNPs, Figure 2). Hence, in serial samples of an individual patient, the same isolate was recovered up

to three months apart, and longer time intervals resulted in the recovery of more diverse isolates of the same serotype.

### **Association of the MSM-outbreak associated lineage with antimicrobial resistance (AMR)**

To investigate the relationship of the MSM-outbreak associated lineage with AMR, the antimicrobial resistance gene (ARG) complement of the isolates was characterised and compared with the time course of the emergence of the lineage (Figure 2, Tables S1, Appendix [B]). Notably, the ARG profile of the MSM-outbreak associated lineage was largely consistent with phenotypic susceptibility data (Table S1) and distinct from the ARG-profile of the Asia- and Africa- associated lineages (Appendix [E]). The resistance profile of the MSM-outbreak associated lineage comprised many near-ubiquitous chromosomal ARGs, and four major mobile genetic elements (MGEs) that each carried multiple ARGs and conferred resistance against various antimicrobials (Table 3, Appendix [B]).

All isolates in the lineage carried a common MGE that encoded resistance to four antimicrobial classes, the *Shigella*-resistance locus multidrug resistance (MDR) element (SRL-MDRE, Figure 2, Table 3). This indicated that the ancestral strain of the lineage was already MDR prior to its introduction into MSM. However, the distribution of other MGEs in the MSM-outbreak associated lineage showed that the lineage acquired additional MGEs (two plasmids and an integron) that encoded resistances to further antimicrobial classes over the time-course of the emergence (Figure 2, Table 3).

Of these MGE acquisitions, one was associated with three apparently ‘successful’ sub-lineages in the MSM-outbreak associated lineage (Figure 2). These prevailing sub-lineages had expanded rapidly and contained the majority (80%, 122 of 154) of the most recently collected isolates (A – C, Figure 2). Isolates from all three sub-lineages contained the plasmid pKSR100 that carried the ARGs *mphA* and *ermB*, and conferred high-level resistance to azithromycin (MICs from 64 to >256mg/L, Table S1). The plasmid had coevolved with, and was mobile within, the lineage (Appendix [C]) and also acquired further ARGs in some

isolates (pKSR100 (Integron) in sub-lineage B isolates, Figure 2, Table 3, Appendix [B - D]). The association of this plasmid with successful sub-lineages suggests a specific selective advantage for resistance against the macrolide azithromycin. Notably, the only chromosomal gene enriched in these sub-lineages was *mdtE*, an efflux pump component that confers resistance to the related macrolide erythromycin (Appendix [B], Table S1).

## DISCUSSION

Here we identified the rapid intercontinental spread of a diarrhoeal pathogen through sexual transmission. Sampling *S. flexneri* serotype 3a isolates from an outbreak in the United Kingdom and contextualising this with smaller numbers of samples from other (low-risk and high-risk) regions, we identified a lineage of *S. flexneri* 3a transmitting endemically in traditionally low-risk regions within a backdrop of genomic epidemiology consistent with our understanding of *S. flexneri* as a travel-associated diarrhoeal disease in these areas. Although further investigation of the potential link to Latin America is warranted, epidemiological evidence linked this lineage with MSM, where international travel likely facilitated global dissemination of the pathogen in less than 20 years. These findings indicate that regional trends in *Shigella* strain replacement and AMR are likely rapidly translatable to the global scale, and highlight the need to consider sexual transmission in the investigation and management of diarrhoeal disease, particularly in the MSM patient group.

Considering the infectivity of *Shigella*, there was remarkably little evidence of transmission of the outbreak lineage to either heterosexual men or women in traditionally low-risk regions, making it pertinent to consider factors specific to MSM that propagate this pathogen. Two such factors were identified here, with the first being transmission-facilitating behaviour. Oro-anal contact was reported by patients from the UK and Canada, and interviewees reported other sexual practices likely to facilitate transmission including high numbers of sexual partners, rare condom use, fisting, scat play and attending sex parties.<sup>9, 16</sup> The second factor is a potentially atypical course of infection in some patients, possibly resulting from

HIV coinfection, a known risk factor for shigellosis<sup>8</sup> (even among MSM<sup>5</sup>). Here, the phylogenetic relationships of serial isolates from some (n=8) patients suggested some atypical features may exist that result in recirculation of the pathogen within the MSM population. These possibilities include chronic infection, relapsing infection or reinfection with diverse isolates of the same serotype over short time scales (the latter being particularly important as immunity to *Shigella* is generally considered to be serotype-specific<sup>19</sup>). Although further studies are needed to investigate these possibilities, it is possible that such atypical infection features (none of which would be traditionally considered features of shigellosis infection), as well as high rates of transmission-facilitating behaviours, were key factors driving the spread and maintenance of *S. flexneri* 3a in MSM.

The association of successful sub-lineages of the outbreak lineage with a highly-mobile azithromycin resistance-conferring plasmid is consistent with reports of azithromycin-resistant MSM-associated shigellosis cases across the United States, Canada, and Australia.<sup>15, 20, 21</sup> This resistance has obvious implications for clinical management (azithromycin treatment failure has already been reported in the Netherlands<sup>22</sup>) and public health surveillance, including the urgent need to develop suitable clinical susceptibility breakpoints and resistance testing for azithromycin.<sup>20, 21</sup> In addition to these specific and immediate implications for the treatment and management of MSM-associated shigellosis, the association with azithromycin resistance represents a disconcerting evolutionary response to antimicrobial selection pressures in a bacterial pathogen.

In the UK, ciprofloxacin is the primary antimicrobial treatment for shigellosis, meaning that fluoroquinolone resistance (as reported in contemporary MSM-associated *S. sonnei* outbreaks<sup>23</sup>) would appear to be advantageous for the pathogen. However, rather than fluoroquinolone resistance being observed in this lineage (Tables S1, Appendix [E]), the prevailing sub-lineages were associated with resistance to azithromycin. Azithromycin is a first or second line treatment for multiple STIs that were co-reported during interview by patients in this study, including gonococcal urethritis, syphilis and chlamydial infections<sup>9, 16, 24-28</sup>. The frequent treatment of MSM-associated shigellosis patients with antimicrobials was

described in a recent study where 43% of cases had received treatment in the previous six months.<sup>23</sup> It is plausible that this selective pressure from azithromycin treatment for co-morbid infections, particularly combined with potential recirculation of *S. flexneri* within this population, resulted in the selection for resistance in this non-target pathogen.

Although unique in its diarrhoeal nature, the rapid spread of this drug resistant pathogen through sexual transmission across an immense geographical area in MSM is perhaps unsurprising given that this has been similarly observed for gonorrhoea, HIV, hepatitis C virus, LGV, and syphilis.<sup>29-33</sup> This repetition underlines the need to identify common factors in this patient community contributing to the public health crisis of antimicrobial resistant STIs in a globalised MSM population. Although comprehensive identification of factors that predispose this population to spread and maintain these pathogens is beyond the scope of this study, factors identified for individual pathogens are likely translatable to other diseases. This is particularly the case with shigellosis where the low infectious dose and severe disease syndrome make it probably only a highly visible ‘tip of the iceberg’ of enteric pathogen transmission in MSM. As well as highlighting the need to rethink enteric pathogens in MSM, this study highlights an unintended impact of antimicrobial treatment on other pathogens, and the rapidity with which pathogens carrying resistance to frontline antimicrobials can spread over large geographical areas in this population.

### **Research in context**

**Evidence before the study** In addition to an existing library of articles on shigellae evolution and bacteriology, the following literature searches were used in PubMed and Scopus: (shigell\* and [homosexual or MSM or gay or ‘men who have sex with men’]), ([homosexual\* or MSM\* or gay or ‘men who have sex with men’] and [cause or aetiology or eitiology] and [diarrhoea\* or gastroent\*]), and (shigell\* and [MSM OR homosexual] and [STI or STD or sexually transmitted]) and full articles were downloaded and reviewed.



**Added value of this study** Using clinical data and whole genome sequencing of pathogens, this study characterised an emergent strain of *Shigella flexneri* 3a causing a UK-wide outbreak that was detected epidemiologically, and included features of atypical infection. Combining the UK samples with those from many other countries, we unequivocally distinguished this sexually transmitted outbreak from travel-acquired cases, and demonstrated that this pathogen had transmitted intercontinentally in men-who-have-sex-with-men. Using phylogenetic inference, we demonstrate that this antibiotic-resistant enteric infection has spread to all inhabited continents traditionally considered low-risk for shigellosis in less than 20 years. By defining the mechanisms and time-course of antibiotic resistance emergence in this pathogen we demonstrate that its resistance is likely an unintended consequence of using antimicrobial treatment for other pathogens in MSM.

**Implications of all the available evidence** This study highlights an emerging sexually transmitted enteric infection in MSM that has spread globally and is resistant to frontline antimicrobials. Building on previous evidence, it indicates that sexual transmission and atypical infection patterns should be considered in the management of shigellosis patients, alongside antimicrobial sensitivity testing. From a public health perspective it highlights the need to validate and implement routine susceptibility testing for azithromycin, and the need to develop a holistic approach for the management of sexually transmitted (and transmissible) infections in MSM. Future research should explore the evidence for chronic *Shigella* infection, particularly investigating the impact of HIV coinfection. The broader burden of sexually transmitted enteric pathogens in MSM should also be quantified, alongside investigations to quantify the exchange and spread of antimicrobial resistance characteristics among these bacteria.

### **Contributors**

KSB designed study, performed data collection, curation and analysis, and wrote manuscript. NRT designed study, contributed to data analysis and wrote manuscript. SRH and JP contributed to data analysis and wrote manuscript. FXW contributed to sample collection,

culture, antibiotic sensitivity testing, contributed to the analysis of mechanisms of resistance and reviewed, critiqued and offered comments on the text. BH performed resistance analysis, sequencing data and contributed to manuscript preparation. TT performed genome sequencing of isolates. MV characterised isolates and performed resistance testing. JDB contributed to the collection of data. AMS was responsible for strains, DNA and data collection for *Shigella* isolated in South Africa, interrogating the database and deciding on which strains would be selected, organizing extraction and shipment of genomic DNA and edited the manuscript. KHK developed surveillance and collected South African strains, was responsible for phenotypic characterization and collecting associated metadata, and edited the manuscript. MG contributed to sample collection, culture, antibiotic sensitivity testing and edited manuscript. NKP contributed to study design and data collection. PA contributed to data analysis. VGA and SZ collected and analysed data and reviewed manuscript. CJ contributed to data interpretation, study design and writing. TD contributed to data interpretation, analysis, study design and writing. PC contributed to collection of UK sexual orientation data, epidemiological data interpretation and writing. VS contributed to data collection, analysis and writing. GH and VG provided behavioural information on UK cases and contributed to the epidemiological interpretation of the UK epidemic and its broader context. GH and VG read and provided critical review of the manuscript. TC was involved in the study design, selection of samples, management of sample shipping and sequencing, metadata collection and curation and sample quality control and also contributed to the analysis and data interpretation. MD performed and interpreted antimicrobial resistance testing for UK isolates. PH contributed to data collection and supplied isolates for testing. SF and KT isolated, confirmed and characterized all the Bangladesh strains used in this study and prepared DNAs for studies. SB and DTP contributed to data collection, analysis and writing the manuscript.

### **Conflicts of interest**

We declare that we have no conflicts of interest. KSB, NRT, SRH, and JP report grants from the Wellcome Trust during the conduct of the study. JP declares non-financial support from Illumina, Inc. outside the submitted work.

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1 **Table 1.** Origin of isolates included in this study (see also methods, Table S1)

2

<b>Sample isolated in</b>	<b>Patient travel history</b>	<b>Country/Region</b>	<b>Date(s)</b>	<b>Sampling strategy</b>	<b>Number</b>
Low risk region	Non-suspect travel histories	UK	2004 - 2013	Representative	179
		France	2010 - 2013	Representative	17
		Australia	2007 - 2014	Selective	30
		Canada	2013	Selective	5
	Suspect travel history (to)	Africa	2009 - 2013	Representative	23
		Asia	2009 - 2013	Representative	19
		Latin America	2010	Representative	2
High-risk region	NA	Africa	2009 - 2011	Representative	14
		Asia	1995 - 2010	Representative	33
		Latin America	2008 - 2010	Representative	5
N/A (outgroup)	-	-	-	-	4
<b>Total</b>					<b>331</b>

3

4 **Table 2. Characteristics and demography of *Shigella flexneri* serotype 3a affected patients under study (proportion of total isolates)**

5

<b>Characteristic</b>	<b>Country/Region</b>	<b>UK</b>	<b>France</b>	<b>Canada</b>	<b>Australia</b>	<b>Africa (South Africa)</b>	<b>Asia (Bangladesh and Vietnam)</b>	<b>Latin America (French Guainía)</b>
Gender	M	195 (94%)	19 (63%)	5 (100%)	30 (91%)	6 (43%)	12 (36%)	2 (40%)
	F	12 (6%)	11 (37%)		3 (9%)	8 (57%)	17 (52%)	2 (40%)
	ND						4 (12%)	1 (20%)
Age (years)	0 - 14	0	2 (7%)			5 (36%)	23 (70%)	
	15 - 59	196 (95%)	17 (57%)		9 (27%)	9 (64%)	5 (15%)	4 (80%)
	60 - 75	8 (4%)	5 (17%)				1 (3%)	
	ND	3 (1%)	6 (20%)	5 (100%)	24 (73%)		4 (12%)	1 (20%)
Travel History	No recent travel	95 (46%)	10 (33%)		1 (3%)			
	No travel history recorded	75 (36%)	1 (3%)	5 (100%)	29 (88%)	14 (100%)	33 (100%)	5 (100%)
	Recent travel to low-risk region	28 (14%)	6 (20%)					
	Recent travel to high-risk region	9 (4%)	13 (43%)		3 (9%)			
<b>Total number of isolates</b>		207	30	5	33	14	33	5

ND denotes not determined.

6

7 **Table 3.** Mobile genetic elements and associated resistances of the MSM-associated outbreak  
 8 lineage

Mobile Genetic Element	Antimicrobial resistance gene(s)	Associated resistances
Shigella Resistance Locus - Multidrug Resistance Element (SRL-MDRE)	<i>bla</i> <sub>OXA-1</sub>	Beta-lactams
	<i>catA1</i>	Chloramphenicol
	<i>aadA1</i>	Aminoglycosides
	<i>tet(B)</i>	Tetracyclines
pKSR100 (Conjugative R- plasmid)	<i>erm(B)</i>	Macrolides (erythromycin)
	<i>mph(A)</i>	Macrolides (azithromycin)
	<i>bla</i> <sub>TEM</sub>	Beta-lactams
pSKR100 Integron	<i>dfrA17</i>	Trimethoprim
	<i>sul1</i>	Sulfonamides
	<i>aadA5</i>	Aminoglycosides
pCERC1 (R-plasmid)	<i>dfrA14</i>	Trimethoprim
	<i>sul2</i>	Sulfonamides
	<i>strA</i>	Aminoglycosides
	<i>strB</i>	Aminoglycosides

9

10

11 **Figure 1. MSM-associated *S. flexneri* 3a in context.** (A) Evolutionary relationships of the  
12 isolates in this study. Tracks adjacent to the phylogenetic tree tip show the traditional  
13 shigellosis risk (coloured as in the map, bottom left, where low-risk regions are blue and high-  
14 risk regions are alternately coloured) of the region of isolate collection (Origin) and regions to  
15 which the patient had recently travelled (Travel). The summarised lineage names are shown  
16 adjacent to tracks. (B) Frequency histogram of patient age for isolates from each of the three  
17 phylogenetic lineages defined in A. (C) Proportion of isolates in each lineage by patient  
18 gender and geography (broken down by travel history for samples from low-risk regions).

19

20 **Figure 2. Genomic portrait of the MSM-associated outbreak lineage of *S. flexneri* 3a.**  
21 Dated phylogenetic tree of MSM-associated outbreak lineage isolates since the lineages  
22 emergence c. 1998. Tree tips are aligned with date of isolate collection according to the  
23 overlaid time grid, and tracks adjacent to tips are coloured to show aspects of patient  
24 geography (Country) and isolate pairing (Patient pairs, coloured similarly according to key  
25 labeled with number of intervening days). Subsequent tracks show the presence (red) and  
26 absence (blue) of antimicrobial resistance-carrying mobile genetic elements (detailed in Table  
27 3). Prevailing sub-lineages are indicated by red markers overlaying the defining internal node  
28 and labeled A – C.

29

30 **Additional information**

31

32 **Table S1.** Full details of isolates used in this study.

33 **Appendix.** Extra material A – E

## References

1. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; **379**(9832): 2151-61.
2. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013; **382**(9888): 209-22.
3. Livio S, Strockbine N, Panchalingam S, et al. Shigella isolates from the Global Enteric Multicenter Study (GEMS) Inform Vaccine Development. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014.
4. Borg ML, Modi A, Tostmann A, 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* 2012; **17**(13).
5. Aragon TJ, Vugia DJ, Shallow S, et al. Case-control study of shigellosis in San Francisco: the role of sexual transmission and HIV infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007; **44**(3): 327-34.
6. Bader M, Pedersen AH, Williams R, Spearman J, Anderson H. Venereal transmission of shigellosis in Seattle-King county. *Sexually transmitted diseases* 1977; **4**(3): 89-91.
7. Dritz SK, Ainsworth TE, Back A, et al. Patterns of sexually transmitted enteric diseases in a city. *Lancet* 1977; **2**(8027): 3-4.
8. Baer JT, Vugia DJ, Reingold AL, Aragon T, Angulo FJ, Bradford WZ. HIV infection as a risk factor for shigellosis. *Emerging infectious diseases* 1999; **5**(6): 820-3.
9. Gilbert VL, Simms I, Gobin M, Oliver I, Hughes G. High-risk drug practices in men who have sex with men. *Lancet* 2013; **381**(9875): 1358-9.
10. Dritz SK, Back AF. Letter: Shigella enteritis venereally transmitted. *The New England journal of medicine* 1974; **291**(22): 1194.
11. Tauxe RV, McDonald RC, Hargrett-Bean N, Blake PA. The persistence of Shigella flexneri in the United States: increasing role of adult males. *American journal of public health* 1988; **78**(11): 1432-5.
12. Ratnayake R, Allard R, Pilon PA. Shifting dominance of Shigella species in men who have sex with men. *Epidemiology and infection* 2012; **140**(11): 2082-6.
13. Marcus U, Zucs P, Bremer V, et al. Shigellosis - a re-emerging sexually transmitted infection: outbreak in men having sex with men in Berlin. *International journal of STD & AIDS* 2004; **15**(8): 533-7.
14. Okame M, Adachi E, Sato H, et al. Shigella sonnei outbreak among men who have sex with men in Tokyo. *Japanese journal of infectious diseases* 2012; **65**(3): 277-8.
15. Valcanis M, Brown JD, Hazelton B, et al. Outbreak of locally-acquired azithromycin-resistant Shigella flexneri infection in men who have sex with men. *Pathology* 2015; **47**(1): 87 - 8.
16. Gilbert VL, Simms I, Gobin M, Oliver I, Hughes G. P3. 149 High-Risk Drug Practises Associated with Shigella Flexneri Serotype 3A Infections Amongst Men



Who Have Sex with Men (MSM) in England. *Sexually Transmitted Infections* 2013; **89**(A194).

17. Baker KS, Mather AE, McGregor H, et al. The extant World War 1 dysentery bacillus NCTC1: a genomic analysis. *Lancet* 2014; **384**(9955): 1691-7.

18. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology* 2007; **7**: 214.

19. Cohen D, Green MS, Block C, Slepon R, Ofek I. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. *Journal of clinical microbiology* 1991; **29**(2): 386-9.

20. Heiman KE, Karlsson M, Grass J, et al. Notes from the field: Shigella with decreased susceptibility to azithromycin among men who have sex with men - United States, 2002-2013. *MMWR Morbidity and mortality weekly report* 2014; **63**(6): 132-3.

21. Gaudreau C, Barkati S, Leduc JM, Pilon PA, Favreau J, Bekal S. Shigella spp. with Reduced Azithromycin Susceptibility, Quebec, Canada, 2012-2013. *Emerging infectious diseases* 2014; **20**(5): 854-6.

22. Hassing RJ, Melles DC, Goessens WH, Rijnders BJ. Case of Shigella flexneri infection with treatment failure due to azithromycin resistance in an HIV-positive patient. *Infection* 2014.

23. Hoffmann C, Sahly H, Jessen A, et al. High rates of quinolone-resistant strains of Shigella sonnei in HIV-infected MSM. *Infection* 2013; **41**(5): 999-1003.

24. Bignell C, FitzGerald M. UK national guideline for the management of gonorrhoea in adults, 2011. *International journal of STD & AIDS* 2011; **22**(10): 541-7.

25. Kingston M, French P, Goh B, et al. UK National Guidelines on the Management of Syphilis 2008. *International journal of STD & AIDS* 2008; **19**(11): 729-40.

26. Gaudreau C, Ratnayake R, Pilon PA, Gagnon S, Roger M, Levesque S. Ciprofloxacin-resistant Shigella sonnei among men who have sex with men, Canada, 2010. *Emerging infectious diseases* 2011; **17**(9): 1747-50.

27. World Health Organisation. Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva, Switzerland: WHO Document Production Services, 2005.

28. British Association for Sexual Health and HIV. UK National guideline for the management of genital tract infection with Chlamydia trachomatis. BASHH Clinical Effectiveness Group. <http://www.bashh.org>; 2006.

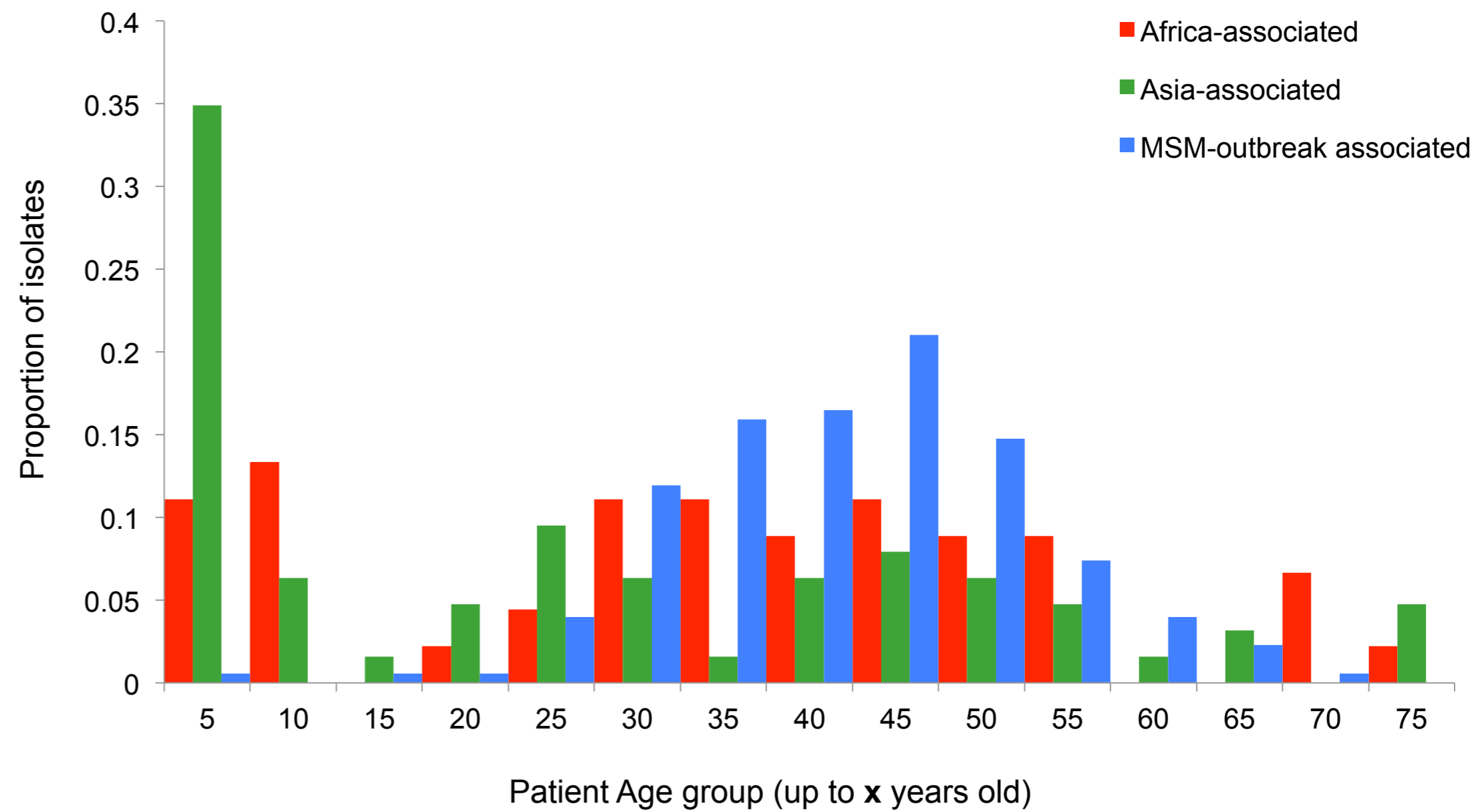
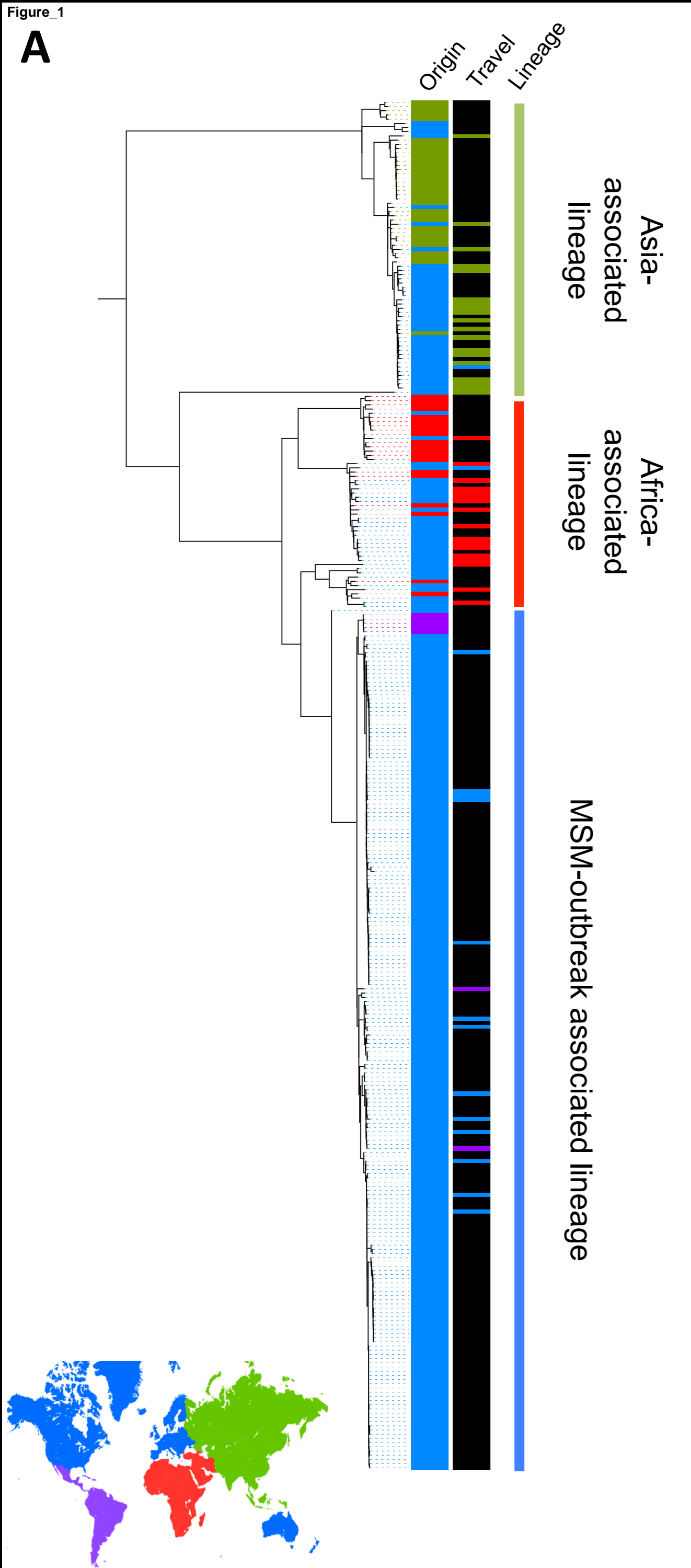
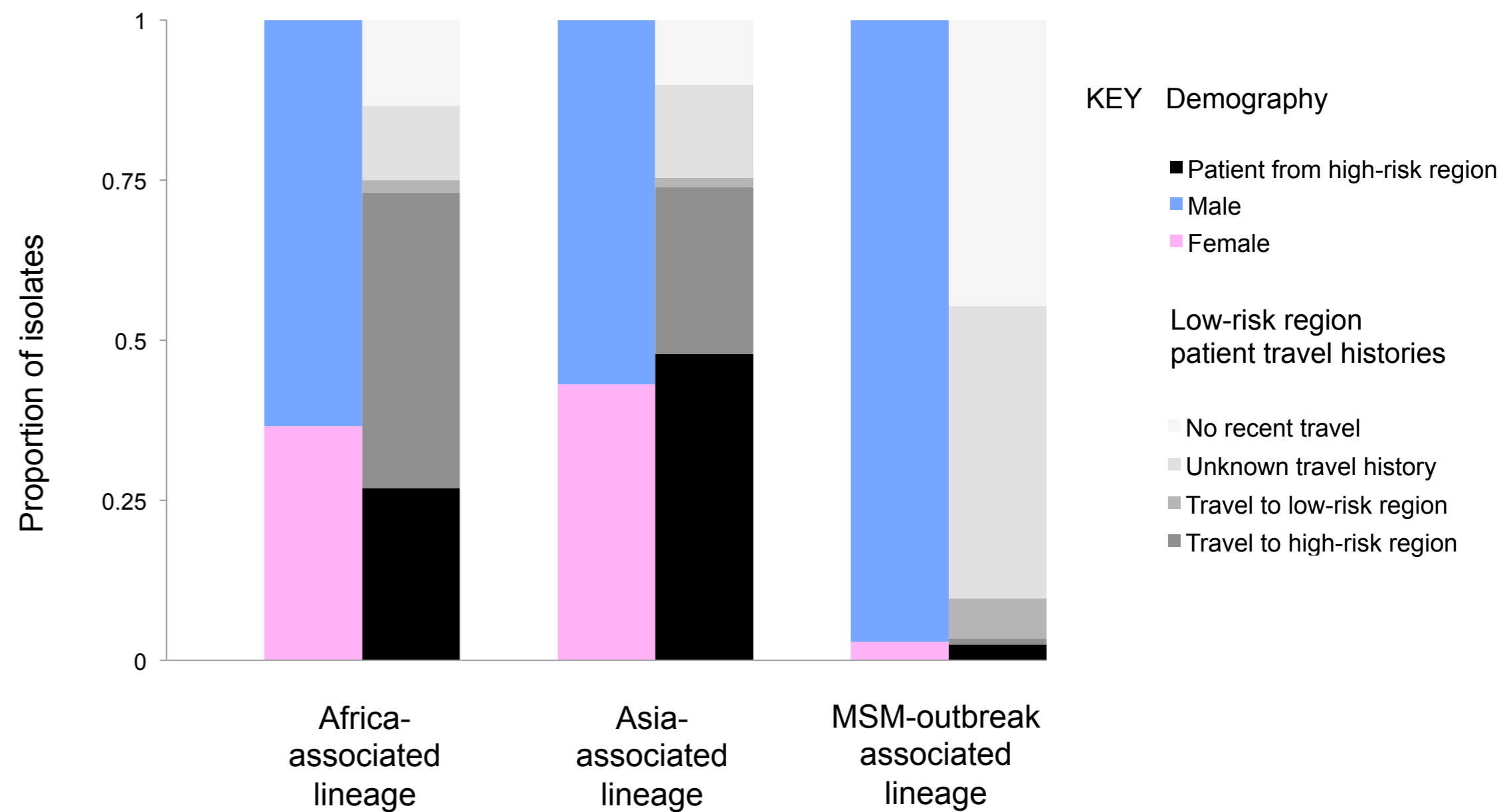
29. Grad YH, Kirkcaldy RD, Trees D, et al. Genomic epidemiology of Neisseria gonorrhoeae with reduced susceptibility to cefixime in the USA: a retrospective observational study. *The Lancet infectious diseases* 2014; **14**(3): 220-6.

30. Nieuwenhuis RF, Ossewaarde JM, Gotz HM, et al. Resurgence of lymphogranuloma venereum in Western Europe: an outbreak of Chlamydia trachomatis serovar I2 proctitis in The Netherlands among men who have sex with men. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2004; **39**(7): 996-1003.

31. Simms I, Fenton KA, Ashton M, et al. The re-emergence of syphilis in the United Kingdom: the new epidemic phases. *Sexually transmitted diseases* 2005; **32**(4): 220-6.

32. van de Laar T, Pybus O, Bruisten S, et al. Evidence of a large, international network of HCV transmission in HIV-positive men who have sex with men. *Gastroenterology* 2009; **136**(5): 1609-17.
33. van Griensven F, de Lind van Wijngaarden JW, Baral S, Grulich A. The global epidemic of HIV infection among men who have sex with men. *Current opinion in HIV and AIDS* 2009; **4**(4): 300-7.

Figure\_1

**A****C****B**

Figure\_2

