

First report of sexually transmitted multi-drug resistant *Shigella sonnei* infections in Switzerland, investigated by whole genome sequencing

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Summary

Shigella sonnei causes foodborne infections, but has recently also been described as a sexually transmitted infection (STI), with increased levels of antimicrobial resistance.

We describe three cases of sexually acquired *Shigella sonnei* infection – the first report of this emerging infection in Switzerland. We used in-house whole genome sequencing (WGS) to investigate possible transmission routes and epidemiological correlations between the three cases. The genomic analysis demonstrated that two of three case isolates were very closely related, with only two single nucleotide polymorphism differences between them, despite being isolated from two unrelated patients at time-points six months apart, and the infections having been acquired at different geographic locations within Europe. All three isolates were found to fall within two of the clusters (1 and 7) defined within UK men who have sex with men (MSM) isolate populations, but with higher divergence, suggesting a more diverse pool circulating within Europe. Phenotypic testing confirmed the genotypic findings, with all three isolates azithromycin resistant, and two out of three resistant to quinolones.

This report underlines the importance of the implementation of new sequencing technologies in the investigation of epidemiological aspects of this STI circulating in the population of MSM. In such cases, therapy should always be guided by antimicrobial susceptibility testing owing to increasing resistances. Greater awareness of this emerging sexually transmitted infection is needed.

Keywords: *Shigella sonnei*, shigellosis, sexually transmitted infections, whole genome sequencing, epidemiological investigation, antimicrobial susceptibility

Introduction

Shigellosis is a well characterised foodborne infection, classically associated with diarrhoea after travel to endem-

ic countries. Infection requires an exceptionally low infectious dose of 10 or fewer bacteria [1]. Since 2001, cases of sexually transmitted *Shigella* spp. among men who have sex with men (MSM) have been reported with increasing frequency, becoming an important public health problem in many countries [2, 3]. Several possible explanations are proposed: changes in sexual practices, such as from oro-genital to direct or indirect oroanal intercourse; practicing chemsex, which disinhibits sexual behaviours [2]; and human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) which may play a role in reducing condom use [4, 5]. Increasing antimicrobial resistance in *Shigella* spp. has also been reported [2, 6, 7].

We report for the first time in Switzerland, three cases of sexually transmitted *Shigella sonnei* infection in MSM, diagnosed in Basel but acquired from three different geographic locations. The isolates were characterised by phenotypic antimicrobial susceptibility testing (AST), and whole genome sequencing (WGS).

Case descriptions

Case 1, November 2016

A 32-year-old male with Centers for Disease Control and Prevention (CDC) A1 stage of HIV infection presented with diarrhoea. One week before the onset of diarrhoea, the patient reported having unprotected passive anal and oral sex during stays in Paris and Munich. The patient is under retroviral therapy with abacavir/lamivudine/dolutegravir. His medical history revealed several episodes of different sexually transmitted infections: secondary syphilis with successful treatment 6 months earlier and a rectal non-lymphogranuloma venereum *Chlamydia trachomatis* infection successfully treated with azithromycin 3 months previously.

Author contributions

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Case 2, May 2017

A 33-year-old male presented to the emergency ward with watery diarrhoea with mucus of 8 days duration, and nausea. The frequency of bowel movements was 10 to 20 times a day, reduced to 5 times per day through self-administered loperamide. Two weeks before, he had travelled to Berlin and visited a club with, according to the patient, a known risk of *Shigella* infection. On clinical examination, the peristaltic sounds were regular, the patient had no abdominal pain and was afebrile. As the patient stabilised and his general condition improved, no antibiotic therapy was initiated.

Case 3, May 2017

A 25-year-old male presented to the emergency ward complaining of profuse diarrhoea of 5 days duration, which had turned bloody 1 to 2 days earlier. He had recently been exposed to risk situations due to unprotected active anal and oral sex in Gran Canaria, but denied engaging in chemsex. During the previous month, the patient had received post-exposure prophylaxis against HIV consisting of tenofovir/emtricitabine/dolutegravir over 4 weeks. The patient was empirically prescribed oral ciprofloxacin 500 mg twice daily and probiotic Bioflorin® three times daily for 5 days, after which symptoms resolved.

Consent for publication

Informed consent was obtained from two of the three patients. The third was “lost to follow up” as he moved out of Switzerland.

Materials and methods

Microbiological analysis

S. sonnei isolates were identified by performing Vitek®2 GN ID (bioMérieux, Marcy-l'Étoile, France) on red colonies growing on BD™ XLD agar (Xylose-Lysine-Desoxycholate Agar; BD, Allschwil, Switzerland), confirmed by Wellcolex™ Colour Shigella agglutination (Thermo Fisher Scientific, Waltham, MA, USA). Minimum inhibitory concentrations (MICs) for an extensive panel of antimicrobials were determined with Etest™ strips (bioMérieux, Marcy-l'Étoile, France) and EUCAST [8] breakpoints used for the interpretation of MICs, except for tetracycline and doxycycline which were interpreted according to CLSI [9].

Genomic analysis

The genomes of the three isolates were sequenced as described [10], with seven unrelated clinical isolates, each providing over 50-fold coverage. Phylogenetic analysis in CLC Genomics Workbench v 9.5.3 mapped against reference strain Ss046 chromosome (accession CP000038), variants called at 10 × minimum coverage, 10 minimum count and 70% minimum frequency. Single nucleotide polymorphism (SNP) tree generation used a neighbour-joining method: minimum coverage 10, minimum coverage 10%, minimum z-score 1.96, multinucleotide variants included. ResFinder was used to identify genomic antibiotic resistance determinants [11].

Results

Phylogenetic analysis shows that two isolates (cases 1 and 2) were very closely related, differing by only two single nucleotide polymorphisms (SNPs), Gly-Ala replacement at codon 99 of a ribonucleoside hydrolase and a premature stop codon within a hypothetical protein. The third isolate (case 3) diverged from these by approximately 340 SNPs. All ten Swiss sequenced isolates belonged to the globally dispersed lineage III [12]. The case isolates fell within the previously identified MSM-related UK clusters [13], with case 1 and 2 isolates 16 SNPs from cluster 7, and case 3 isolate 12 SNPs from UK cluster 1 (fig. 1).

Genomic antibiotic resistance determinants concur with the phenotypic results (table 1). All three case isolates possessed conjugative plasmid pKSR100 [6], which is found only in MSM lineages (fig. 1), and carries resistance determinants *erm(B)* and *mph(A)* for macrolides, and *bla*_{TEM-1} for β-lactams. Isolates from cases 1 and 2, unlike the rest of cluster 7, carried this plasmid, as well as the pKSR100 integron, with *dfrA17* for trimethoprim, *sul1* for sulphonamide and *aadA5* for spectinomycin resistance. The case 3 isolate, like all other cluster 1 isolates, carried a plasmid similar to the conjugative pEC3I [15] with *dfrA1* for trimethoprim, *sul2* for sulphonamide and *aadA1* for streptomycin and spectinomycin resistance. Plasmid pSpA was found in cases 1 and 2 and all cluster 7 isolates, and is otherwise stochastically distributed in the phylogeny, carrying *strAB* for streptomycin, *sul2* for sulphonamide and *tet(A)* for tetracycline resistance. The isolate from case 3 carried a chromosomal *tet(B)*, and case 1 and 2 isolates carried quinolone resistance mutations: S80I in ParC and S83L and D87G in GyrA. The 214 kb virulence plasmid which characterises *Shigella*, pSS_046, was not identified in any of the case isolates (fig. 1), but is known to be unstable during culturing and is often absent from whole genome data [12].

Discussion

WGS provides highly discriminatory molecular typing for investigating outbreaks of *Shigella* spp. [13]. Two of the isolates (cases 1 and 2) were very closely related, with only two SNP differences between them. However, they are unlikely to have resulted from direct transmission as: the case dates were more than 6 months apart; they were assumed to have been acquired at two different geographic locations (Berlin and Paris/Munich); and the estimated substitution rate of *S. sonnei* genomes is 2.2 SNPs per chromosome per year [12].

MSM-associated lineages have been defined within *Shigella flexneri* serotype 3a (with isolates from the UK, France, Canada and Australia); *S. flexneri* serotype 2a (UK, France) and *S. sonnei* (UK, France), displaying higher than normal levels of antimicrobial resistance related to the presence of specific mobile elements including pKSR100 [6, 14]. The cases found in Switzerland are related to MSM clusters identified in the UK [13], although with more genomic diversity, suggesting a more diverse pool circulating more widely within European MSM communities. Very recently published genomes from the UK and France extend this pool of diversity [14], but are not

more closely related to Swiss cases than those described here (data not shown).

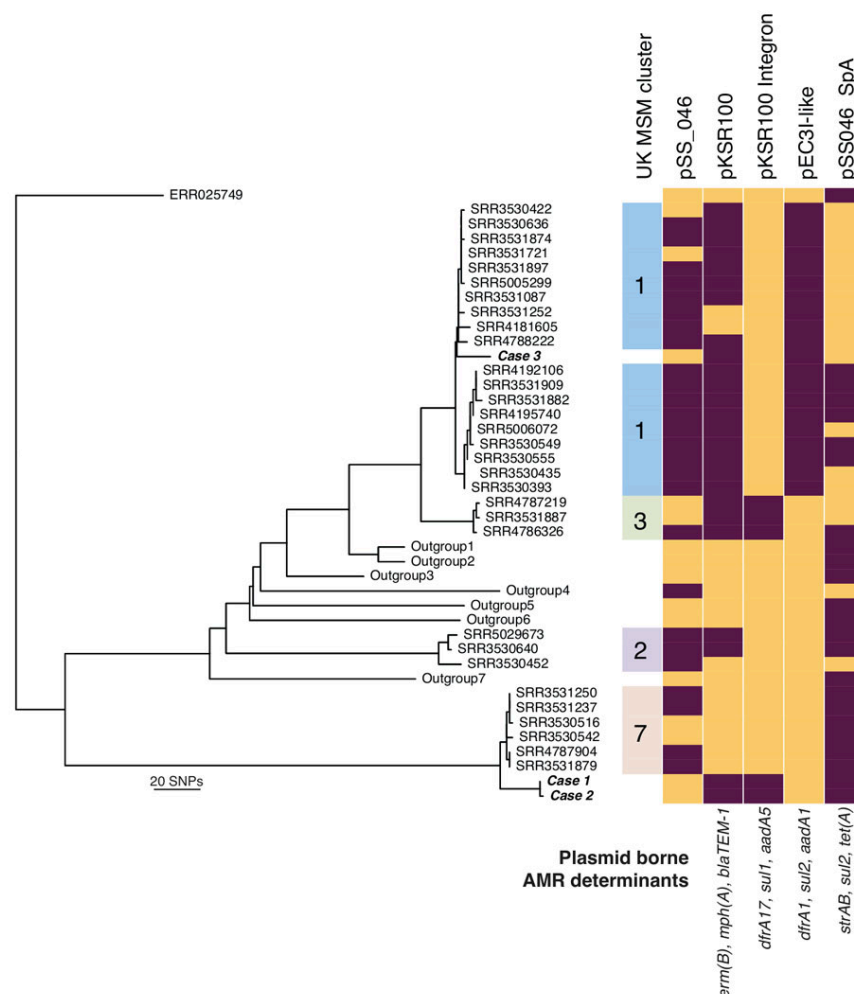
The epidemiology of shigellosis in the MSM population in Europe is largely unexplored, because of lack of surveillance and reporting on this issue. Detailed data are only available from the UK [6, 7, 13, 14], which has led to targeted public awareness campaigns, and may be responsible for the observed drop in *Shigella* cases among the MSM group in the latest reports (2016) [16]. Our report details the first European cases that we are aware of, outside the UK and France.

Plasmids, especially self-mobile conjugative plasmids, are involved in increasing antimicrobial resistance. All three case isolates carried pKSR100, previously identified in MSM-related *S. flexneri* [6], which carries *mph(A)* encoding a macrolide-inactivating 2'-phosphotransferase, ex-

plaining the very high azithromycin MICs (table 1). Selective pressure to gain and retain this plasmid may come from single dose azithromycin injections for genital chlamydia, which could be subtherapeutic for invasive shigellosis. Isolates from cases 1 and 2 carried chromosomal quinolone resistance mutations, which also confirmed phenotypic AST findings. The identified *aadA1*, *aadA5* and *strAB* genes confer resistance to spectinomycin and streptomycin, but not to other aminoglycosides such as tobramycin and amikacin which, as expected, tested susceptible in all three case isolates.

The current Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and management of infectious diarrhoea recommend ciprofloxacin and azithromycin as first choice and trimethoprim-sulphamethoxazole or ampicillin as second choice for non-in-

Figure 1: *S. sonnei* chromosome phylogeny and presence of key plasmids. The phylogenetic analysis was performed in CLC Genomics Workbench v. 9.5.3, mapping against reference strain Ss046 chromosome (accession number CP000038), variants called at 10 × minimum coverage, 10 minimum count and 70% minimum frequency. SNP tree generation used the following parameters: minimum coverage = 10, minimum coverage % = 10, minimum z-score = 1.96, MNVs (multinucleotide variants) included, and used a neighbour-joining method. Lineage III sample ERR025749 [12] is an outgroup. UK MSM isolates (beginning SRR) [13] defined clusters 1, 2, 3, and 7. Clusters 1 and 3 were re-defined as *S. sonnei* MSM sublineage 4 in [14]. Within cluster 1, 37 SNPs were identified, and 22 within cluster 7. SNPs between case 1 and 2 isolates cause Gly-Ala replacement at codon 99 of a ribonucleoside hydrolase, and a premature stop codon within a hypothetical protein. Unrelated clinical *S. sonnei* isolates are included for context ("outgroup"). To the right of the phylogenetic tree, aligned with the isolates, the clusters numbers of UK MSM isolates are shown, and the presence (purple) or absence (yellow) of plasmids as defined by mapping (pSS_046, CP000039; pSS046_SpA, CP000641; pKSR100, LN624486; pEC3I, KU932021; 53G pB, HE616530; 53G pC, HE616531): considered as present when reads map to over 85% of the length. Antimicrobial resistance determinants carried by each plasmid are also indicated. Figure generated using Phandango.



vasive shigellosis [17]. Based on our findings, and results of other similar studies, the empirical use of quinolones, azithromycin or trimethoprim-sulphamethoxazole should no longer be recommended for shigellosis in the MSM population.

Conclusion

WGS is a valuable tool for epidemiological investigation of shigellosis. Preventive and educational measures in the MSM community are necessary in order to reduce *Shigella* spp. infections in the future. Clinicians need to be aware of this transmission route, and therapy should always be guided according to antimicrobial susceptibility testing.

Read data has been deposited with the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>) under project number PRJEB23646.

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References

- DuPont HL, Levine MM, Hornick RB, Formal SB. Inoculum size in shigellosis and implications for expected mode of transmission. *J Infect Dis.* 1989;159(6):1126–8. doi: <http://dx.doi.org/10.1093/infdis/159.6.1126>. PubMed.
- Chiou CS, Izumiya H, Kawamura M, Liao YS, Su YS, Wu HH, et al. The worldwide spread of ciprofloxacin-resistant *Shigella sonnei* among HIV-infected men who have sex with men, Taiwan. *Clin Microbiol Infect.* 2016;22(4):383.e11–6. doi: <http://dx.doi.org/10.1016/j.cmi.2015.12.021>. PubMed.
- Simms I, Field N, Jenkins C, Childs T, Gilbert VL, Dallman TJ, et al. Intensified shigellosis epidemic associated with sexual transmission in men who have sex with men--*Shigella flexneri* and *S. sonnei* in England, 2004 to end of February 2015. *Euro Surveill.* 2015;20(15):21097. doi: <http://dx.doi.org/10.2807/1560-7917.ES2015.20.15.21097>. PubMed.
- Chen YH, Snowden JM, McFarland W, Raymond HF. Pre-exposure Prophylaxis (PrEP) Use, Seroadaptation, and Sexual Behavior Among Men Who Have Sex with Men, San Francisco, 2004–2014. *AIDS Behav.* 2016;20(12):2791–7. doi: <http://dx.doi.org/10.1007/s10461-016-1357-2>. PubMed.
- Lal L, Audsley J, Murphy DA, Fairley CK, Stooze M, Roth N, et al.; VicPrEP Study Team. Medication adherence, condom use and sexually transmitted infections in Australian preexposure prophylaxis users. *AIDS.* 2017;31(12):1709–14. doi: <http://dx.doi.org/10.1097/QAD.0000000000001519>. PubMed.
- Baker KS, Dallman TJ, Ashton PM, Day M, Hughes G, Crook PD, et al. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. *Lancet Infect Dis.* 2015;15(8):913–21. doi: [http://dx.doi.org/10.1016/S1473-3099\(15\)00002-X](http://dx.doi.org/10.1016/S1473-3099(15)00002-X). PubMed.
- Mook P, McCormick J, Bains M, Cowley LA, Chattaway MA, Jenkins C, et al. ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015. *Emerg Infect Dis.* 2016;22(11):1948–52. doi: <http://dx.doi.org/10.3201/eid2211.160653>. PubMed.
- The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 7.1, 2017. http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 27th edition. CLSI document M1002017. Available at: <http://www.facm.ucl.ac.be/intranet/CLSI/CLSI-2017-M100-S27.pdf>.
- Sommerstein R, Führer U, Lo Priore E, Casanova C, Meinel DM, Seth-Smith HM, et al.; On Behalf Of Anresis; On Behalf Of Swissnos. *Burkholderia stabilis* outbreak associated with contaminated commercially-available washing gloves, Switzerland, May 2015 to August 2016. *Euro Surveill.* 2017;22(49). doi: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.49.17-00213>. PubMed.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother.* 2012;67(11):2640–4. doi: <http://dx.doi.org/10.1093/jac/dks261>. PubMed.
- Holt KE, Baker S, Weill FX, Holmes EC, Kitchen A, Yu J, et al. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. *Nat Genet.* 2012;44(9):1056–9. doi: <http://dx.doi.org/10.1038/ng.2369>. PubMed.
- Dallman TJ, Chattaway MA, Mook P, Godbole G, Crook PD, Jenkins C. Use of whole-genome sequencing for the public health surveillance of *Shigella sonnei* in England and Wales, 2015. *J Med Microbiol.* 2016;65(8):882–4. doi: <http://dx.doi.org/10.1099/jmm.0.000296>. PubMed.

Table 1: Minimal inhibitory concentrations (MICs) of three *S. sonnei* isolates.

Antimicrobial	<i>S. sonnei</i> MIC (mg/l) with interpretation		Case 1		Case 2		Case 3	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Amoxicillin	>256	R	>256	R	>256	R	>256	R
Amoxicillin-clavulanic acid	6	S	6	S	6	S	6	S
Piperacillin-tazobactam	2	S	2	S	2	S	2	S
Ceftazidime	0.064	S	0.064	S	0.064	S	0.047	S
Ceftriaxone	0.032	S	0.023	S	0.023	S	0.023	S
Cefepime	0.032	S	0.023	S	0.023	S	0.023	S
Ertapenem	0.002	S	0.003	S	0.003	S	0.003	S
Imipenem	0.19	S	0.19	S	0.19	S	0.25	S
Meropenem	0.032	S	0.023	S	0.023	S	0.012	S
Tobramycin	0.75	S	0.75	S	0.75	S	0.75	S
Amikacin	3	S	2	S	2	S	2	S
Tetracycline	24	R	24	R	24	R	64	R
Doxycycline	64	R	64	R	64	R	96	R
Trimethoprim-sulphamethoxazole	>32	R	>32	R	>32	R	>32	R
Ciprofloxacin	4	R	6	R	6	R	0.008	S
Levofloxacin	6	R	6	R	6	R	0.032	S
Colistin	1.5	S	1	S	1	S	1.5	S
Azithromycin	>256	R	>256	R	>256	R	>256	R

S = susceptible; R = resistant

- 14 Baker KS, Dallman TJ, Field N, Childs T, Mitchell H, Day M, et al. Horizontal antimicrobial resistance transfer drives epidemics of multiple *Shigella* species. *Nat Commun*. 2018;9(1):1462. Published online April 15, 2018. doi: <http://dx.doi.org/10.1038/s41467-018-03949-8>. PubMed.
- 15 Mattila S, Ruotsalainen P, Ojala V, Tuononen T, Hiltunen T, Jalasvuori M. Conjugative ESBL plasmids differ in their potential to rescue susceptible bacteria via horizontal gene transfer in lethal antibiotic concentrations. *J Antibiot (Tokyo)*. 2017;70(6):805–8. doi: <http://dx.doi.org/10.1038/ja.2017.41>. PubMed.
- 16 England PH. *Shigella* infections in England with a focus on sexual transmission between men who have sex with men: laboratory reports 2004 to 2016. London: Public Health England, 2016.
- 17 Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. *Clin Infect Dis*. 2017;65(12):1963–73. doi: <http://dx.doi.org/10.1093/cid/cix959>. PubMed.