

THE LANCET

Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed.
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Supplement to: Charles H, Prochazka M, Thorley K, et al. Outbreak of sexually transmitted, extensively drug-resistant *Shigella sonnei* in the UK, 2021–22: a descriptive epidemiological study. *Lancet Infect Dis* 2022; published online July 6. [https://doi.org/10.1016/S1473-3099\(22\)00370-X](https://doi.org/10.1016/S1473-3099(22)00370-X).

Appendix

1. Supplementary methods

1.1 DNA extraction, library preparation, Nanopore sequencing and data processing

High-molecular weight (HMW) genomic DNA was extracted and purified using the Revolugen Fire Monkey HMW DNA extraction kit (RevoluGen, UK), and DNA for each extract was quantified using a Qubit and the HS (high sensitivity) dsDNA assay kit (ThermoFisher Scientific, Waltham, USA), as previously described ^[1,2]. Library preparation and sequencing was performed as previously described² before sequencing on the MinION (Oxford Nanopore Technologies, Oxford, UK) for 72h.

Data produced in a raw FAST5 format was base-called and de-multiplexed using Guppy v5.0.17 FAST model (Oxford Nanopore Technologies, Oxford, UK) into FASTQ format. De-multiplexing was performed using Deepbinner v0.2.0 ^[3], sequencing run metrics were generated using Nanoplot v1.8.1^[4], read trimming was performed using Porechop v0.2.4 ^[5] (Wick RR, <https://github.com/rrwick/Porechop>) and finally, read filtering using Filtrlong v0.2.0 ^[6] (Wick RR, <https://github.com/rrwick/Filtrlong>) as previously described ^[1,2].

1.2 De novo assembly, correction, re-orientation and annotation

The filtered nanopore FASTQ file with the 50x coverage of longest reads were assembled using Flye v2.8 ^[7] with the minimum overlap length (-m) set to 10000. Correction (polishing) of the assemblies was performed in a three-step process as previously described ^[1,2]. Firstly, using Nanopolish v0.13.2 ^[8], secondly, using Pilon v1.23 ^[9] and finally Racon v1.4.20 ^[10] as previously described ^[1,2]. All Nanopore read alignments were generated with Minimap2 v2.22 ^[11] and Samtools v1.10 ^[12] and short-read alignments generated with BWA MEM v0.7.17 ^[13] and Samtools v1.10 ^[12]. As the chromosome from each assembly was circularised and closed, they were re-orientated to start at the *dnaA* gene (GenBank accession no. NC_000913) from *E. coli* K-12, using the --fixstart parameter in Circlator v1.5.5 ^[14]. Prokka v1.13 ^[15] was used to annotate the final assemblies.

1.3 In silico plasmid typing and AMR determinant detection

In silico identification of AMR genes among the isolates in this study was performed using AMRFinderPlus v3.10 ^[16]. The plasmid replicon for each non-chromosomal contig within each sample's final assembly was compared to PlasmidFinder's v2.1 Enterobacteriaceae reference database ^[17]. An alignment was generated using Easyfig v2.2.5 ^[18] relying on BLASTn v2.9 ^[19]. BLASTn parameters used were minimum identity = 90% and minimum length hit = 100bp.

1.4 Data deposition

Illumina and Nanopore FASTQ files are available from National Center for Biotechnology Information (NCBI) BioProject PRJNA315192. The SRA (sequence read archive) accession numbers for both technologies are in Supplementary Table 1.

Supplementary Table 1: Publicly available accessions for samples that underwent long read sequencing using Oxford Nanopore Technology (ONT)

Sample ID	BioProject accession	BioSample accession	Nanopore SRA accession
1324211	PRJNA315192	SAMN20849361	SRR18254047
1502196	PRJNA315192	SAMN22657138	SRR18254048
1519721	PRJNA315192	SAMN22962688	SRR18254049
1519755	PRJNA315192	SAMN22962676	SRR18254050
1527837	PRJNA315192	SAMN23134809	SRR18254051
1527854	PRJNA315192	SAMN23134804	SRR18254052
1534306	PRJNA315192	SAMN23441105	SRR18254053
1538007	PRJNA315192	SAMN23569621	SRR18254054
1538717	PRJNA315192	SAMN23605158	SRR18254055

1.5 Hyperlinks to EUCAST and CLSI criteria

EUCAST criteria can be found at <https://www.eucast.org/> (last accessed 26/04/2022)

CLSI criteria can be found at <https://clsi.org/> (last accessed 26/04/2022)

2. Bespoke outbreak questionnaire

CONFIDENTIAL – XDR *Shigella sonnei* questionnaire

First name:	Surname:
Date of birth:	
GDW sample date:	GDW identifier:
HPT:	HPZone number:
Interviewer initials:	Interview date:

INFORMATION ABOUT STUDY SPOKEN TO PATIENT BEFORE INTERVIEW

- Good morning/afternoon/evening. Am I speaking to *[insert full name]*?
- I am a *[scientist/doctor/public health officer]* working closely with the NHS to track diseases and investigate outbreaks.
- For identification, could you please confirm your date of birth with me? *[check it matches to verify you are speaking to the right person]*
- I'm calling because we were informed you had a gastrointestinal infection with *Shigella* in *[insert month and year of specimen date]*. Our laboratories have done some additional tests and we believe your infection may be caused by a possible type of *Shigella* that is increasing across the UK.
- We need a bit more information from you to understand this outbreak better, is this a good time to talk? This will take a maximum of 20 minutes.
- This is a bacteria that causes gut infection resulting in symptoms like diarrhoea, fever, and abdominal pain. It can be transmitted from person to person, or from eating contaminated food or drinks.
- We are currently investigating this to better understand how the infection is being transmitted, the severity of the illness and the treatments that help with the illness so that measures can be taken to prevent more people from becoming ill.
- We do this by interviewing everyone that has recently had this type of *Shigella* infection and hoped that you would be able to spare 20 minutes or so to answer some questions. Your contribution is very important in helping us understand more about this infection. Would you agree to help us in this investigation?
- Thank you for agreeing to participate.

- Since *Shigella* can be caught from direct contact between people, such as during sex, we will be discussing some personal questions, but if there is anything you do not want to answer, please just say so; we would appreciate it if you could be as honest as possible.
- Please know that this is a confidential conversation, any information you provide will be handled in line with the Data Protection Act.

SECTION 1: PERSONAL BACKGROUND

We will first ask you some questions to understand your personal background:

1. Where do you currently live? _____ Postcode:

2. What is your country of birth?

3. How would you describe your ethnicity?

White	Mixed	Asian	Black	Other
<input type="checkbox"/> British	<input type="checkbox"/> White & Black Caribbean	<input type="checkbox"/> Indian	<input type="checkbox"/> British	<input type="checkbox"/> Hispanic or Latinx
<input type="checkbox"/> Irish	<input type="checkbox"/> White and Black African	<input type="checkbox"/> Pakistani	<input type="checkbox"/> Caribbean	<input type="checkbox"/> Chinese
<input type="checkbox"/> Other	<input type="checkbox"/> White and Asian	<input type="checkbox"/> Bangladeshi	<input type="checkbox"/> African	<input type="checkbox"/> Arab
	<input type="checkbox"/> Other	<input type="checkbox"/> Other	<input type="checkbox"/> Other	<input type="checkbox"/> Another way _____

4. How would you describe your sexual orientation?

- Gay/lesbian
- Straight
- Bisexual
- Other:

5. How would you describe your gender?

- Man (including trans man)
- Woman (including trans woman)
- Non-binary
- In another way: _____

6. What sex were you assigned at birth?

- Male
- Female
- Intersex

7. What is your main occupation or activity?

- | | Yes | No |
|--|--------------------------|--------------------------|
| a. Food handler preparing or serving unwrapped ready-to-eat food/drinks | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Clinical, social care or nursery staff working with vulnerable people | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Other: _____ | | |

8. Were you in any of the following locations in the ONE WEEK prior to your illness?

- | | Yes | No |
|---------------------------|--------------------------|--------------------------|
| a. London | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Manchester | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Other places in the UK | <input type="checkbox"/> | <input type="checkbox"/> |

d. Overseas

9. Please detail places/locations where you travelled (*leave blank if no travel history*)

SECTION 2: EXPERIENCE OF ILLNESS AND CARE

We will now ask you some questions about your recent illness:

10. What symptoms did you have?

- | | Yes | No |
|-------------------------------|--------------------------|--------------------------|
| a. Diarrhoea | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Vomiting | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Blood in stools | <input type="checkbox"/> | <input type="checkbox"/> |
| d. Fever (raised temperature) | <input type="checkbox"/> | <input type="checkbox"/> |
| e. Abdominal pain (cramps) | <input type="checkbox"/> | <input type="checkbox"/> |
| f. Fatigue | <input type="checkbox"/> | <input type="checkbox"/> |
| g. Other: _____ | | |

11. When did your symptoms start and end? (if not sure, it's OK to give a ballpark)

- a. Start date: _____ b. End date: _____

12. Did you seek help in any of the following services?

- | | Yes | No |
|--------------------------------------|--------------------------|--------------------------|
| a. GP (calling or in-person) | <input type="checkbox"/> | <input type="checkbox"/> |
| b. A&E | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Sexual health services/GUM Clinic | <input type="checkbox"/> | <input type="checkbox"/> |
| d. Ambulance services | <input type="checkbox"/> | <input type="checkbox"/> |
| e. Hospital overnight stay | <input type="checkbox"/> | <input type="checkbox"/> |
| f. Other: _____ | | |

13. If hospitalised/went to A&E, what is the name of the hospital?

14. If hospitalised, how many nights did you stay overnight?

15. From what you know, did you have any of the following complications?

- | | Yes | No | Don't know |
|---------------------|--------------------------|--------------------------|--------------------------|
| a. Sepsis or shock | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Need for surgery | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Other: _____ | | | |

16. From what you know, did you receive any of the following treatments?

- | | Yes | No | Don't know |
|--|--------------------------|--------------------------|--------------------------|
| a. Oral antibiotics | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Intravenous antibiotics (IV medicine in a drip) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Intravenous rehydration (IV fluid in a drip) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| d. Other: _____ | | | |

17. From what you know, tell us more about any antibiotics you received, including their names and how long you received them for (leave blank if no antibiotics were used)

18. Have you had *Shigella* before?

- Yes
- No
- Don't know

19. If yes, could you tell us the month and year when it happened?
(include suspected episodes)

- a. Episode 1
- b. Episode 2
- c. Episode 3

Month	Year

20. Do you have any underlying health conditions that may cause immunosuppression, which could be important for *Shigella*?

- | | Yes | No |
|---------------------------------------|--------------------------|--------------------------|
| a. Living with HIV | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Cancer | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Regular steroid/corticosteroid use | <input type="checkbox"/> | <input type="checkbox"/> |
| d. Recent COVID-19 hospitalisation | <input type="checkbox"/> | <input type="checkbox"/> |
| e. Other: _____ | | |

21. Do you have any other comments about your recent experience with *Shigella*?

SECTION 3: SEXUAL HISTORY

We know one of the ways *Shigella* can be transmitted is through sex, so we will be discussing some personal questions.

22. In the ONE WEEK BEFORE you started having symptoms, did you engage in:

- | | Yes | No | Don't know |
|---|--------------------------|--------------------------|--------------------------|
| a. Sex with new partner (someone you had not had sex with before) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Sex with one-off partner (someone you only are seeing once) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Sex with occasional partner (someone you have sex with sporadically) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| d. Sex with established partners (someone you have sex with often) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| e. Group sex (sex with multiple people at the same time) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| f. Chemsex (use of drugs e.g.: G, crystal meth, mephedrone, during sex) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| g. Sex with partners who are not regular residents in the UK | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| h. Sex in locations different than your usual city/town of residence? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

23. How did you meet these people? (detail any events, venues, or apps listed)

24. Have you had any STIs in the last year? (add any details as needed, including PrEP use)

SECTION 4: IDENTIFICATION OF PROBABLE CASES

We know *Shigella* can be easily transmitted to others, so we want to ask you some questions about whether any of your contacts also have or had *Shigella*. These can be household contacts (someone who lives with you) or sexual contacts (someone you have had sex with).

25. Did any of your contacts have symptoms of *Shigella* (such as diarrhoea, fever, or stomach pain) 1 WEEK BEFORE OR AFTER your contact?

- | | Yes | No | Don't know |
|-----------------------|--------------------------|--------------------------|--------------------------|
| a. Sexual contacts | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| b. Household contacts | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| c. Other: _____ | | | |

We need some general information about these contacts. If they are food handlers or care workers, it is possible someone from our organisation will reach out to you again.

Gender	Type of contact (Household/Sexual) <i>If household: Parent/Sibling/Guardian/Friend</i> <i>If sexual: Established/New/Occasional/One-off/Sex worker</i>	Age	Symptom onset date (approximate)	Risk group*

*Risk groups

Group A: Personal hygiene difficulties (requires help or has unsatisfactory toilet / wash facilities)

Group B: Children aged 5 years and under (i.e. up to 6th birthday) attending childcare facilities

Group C: Food handlers who prepare or serve unwrapped ready-to-eat food (including drink)

Group D: Clinical, social care or nursery staff who work with vulnerable people

SECTION 5: CLOSING REMARKS AND HEALTH PROMOTION

We have now come to the end of the interview. Thank you very much for giving up your time to talk to us and for all your help with answering the questions.

26. After this conversation, how do you think you acquired your recent infection? (add notes based on need and additional comments from the case)

➤ If the infection IS suspected to be sexually transmitted, this is optional messaging to be provided based on need and context:

- *Shigella* is caught from very small amounts of bacteria in poo getting into your mouth during sex, so sex does not have to be “messy” for people to be at risk. However, good hygiene can prevent its transmission. There is a risk from rimming or giving oral sex after anal sex or play, including fingering. To avoid it, wash your hands, bum, and genitals after anal sex. Try to avoid going back to oral sex after anal play has started: the idea is to avoid anything that has been in contact with someone's bum go into your mouth.
- Have you had a sexual health check-up since you had these symptoms? We recommend getting tested for other STIs including HIV, as you might be at risk of other infections. Gay and bisexual men should test annually for HIV and STIs and every 3 months if having condomless sex with new or casual partners.
- If you ever experience symptoms like this again, especially after a sexual contact, you should attend a sexual health clinic or your GP, and tell them that you think this might be sexually transmitted, so that you can be tested for *Shigella*.
- If you are unsure about your HIV status, you can go to a sexual health clinic or a community testing site, ask your GP for an HIV test or request a self-sampling kit online or obtain a self-testing kit
- If you are HIV negative, PrEP (pre-exposure prophylaxis for HIV) might be for you. This can be accessed for free from sexual health services across England.
- You can also protect yourself from hepatitis which damages the liver, and genital warts and some types of cancers by getting vaccinated against HPV, hepatitis A and hepatitis B at your next sexual health service visit.
- Ask your sexual health service where you can get support to reduce risks associated with recreational drug use (including chemsex). You can also use resources on the Harm Reduction Works website to keep themselves safe and reduce health harms.

- Find more information about *Shigella* at Sexwise: www.sexwise.org.uk/stis/shigella
 - If the infection IS NOT suspected to be sexually transmitted, exploring other sources and exposures (food, drink, other person-to-person contact) is needed. This can be done using routine Enhanced Surveillance Questionnaires administered by HPTs.

3. Standard shigellosis questionnaire

The standard shigellosis questionnaire can be found at <https://www.gov.uk/government/publications/shigellosis-public-health-management-and-questionnaire> (last accessed 26/04/2022)

4. Supplementary references

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