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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Bertran M, D'Aeth J C, Hani E, et al. Trends in invasive *Haemophilus influenzae* serotype a disease in England from 2008–09 to 2021–22: a prospective national surveillance study. *Lancet Infect Dis* 2023; published online June 22. https://doi.org/10.1016/S1473-3099(23)00188-3.

Appendix

Trends in invasive Haemophilus influenzae serotype a disease in England from 2008–09 to 2021–22: a prospective national surveillance study

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Section 1 – Additional methodological information

1a) Second generation surveillance system (SGSS)

UKHSA receives electronic laboratory reports through the Second Generation Surveillance System (SGSS) of clinically significant pathogens from all National Health Service (NHS) laboratories. Laboratories with a primary diagnostic role are legally required to report any invasive sample of H. influenzae, under the Health Protection Legislation Guidance 2010. Further information on this legal requirement and the reporting of H. influenzae cases from laboratories can be found at: https://www.gov.uk/guidance/notifiable-diseases-and-causative-organisms-how-to-report#laboratories-report-notifiable-organisms-causative-agents

1b) Laboratory species confirmation and serotyping

Isolates submitted to the UKHSA national reference laboratory were confirmed as *H. influenzae* by their growth requirement for X and V factors, and *ompP2*-specific polymerase chain reaction (PCR) positivity.[1,2] Capsular serotype was determined using slide agglutination (with monovalent Hia–f specific antisera),[1] and PCR (using *bexA*-specific primers,[3] and capsule-specific primers for types Hia–f).[4]

1c) Whole Genomic sequencing, assembly and MLST typing methods

Genomic DNA was extracted from pure *H. influenzae* cultures following overnight growth at 37°C on chocolate blood agar plates. Extraction was performed using the QIAGEN QIAsymphony SP platform and QIAsymphony DSP DNA Mini Kit, using the manufacturer's recommended tissue extraction protocol for Gram negative bacteria (including an overnight pre-incubation with proteinase K in ATL buffer and RNAse A treatment). Genomic DNA was sequenced by the UKHSA's Central Sequencing Laboratory using the Illumina HiSeq2500 and NextSeq1000 platforms to produce deplexed fastq files. Short reads produced from sequencing were trimmed using Trimmomatic[5] and the MLST[6] was derived using MOST,[7] with MLST profiles taken from the *H.* influenzae pubMLST site.[8] Illumina reads were then *de novo* assembled using Shovill v0.9.0, contigs of length <500bp were removed from the assemblies and quality control (QC) was performed using QUAST.[9]

Figure A. Map of Hia cases in England by epidemiological year. Number of cases is shown in brackets as (n).

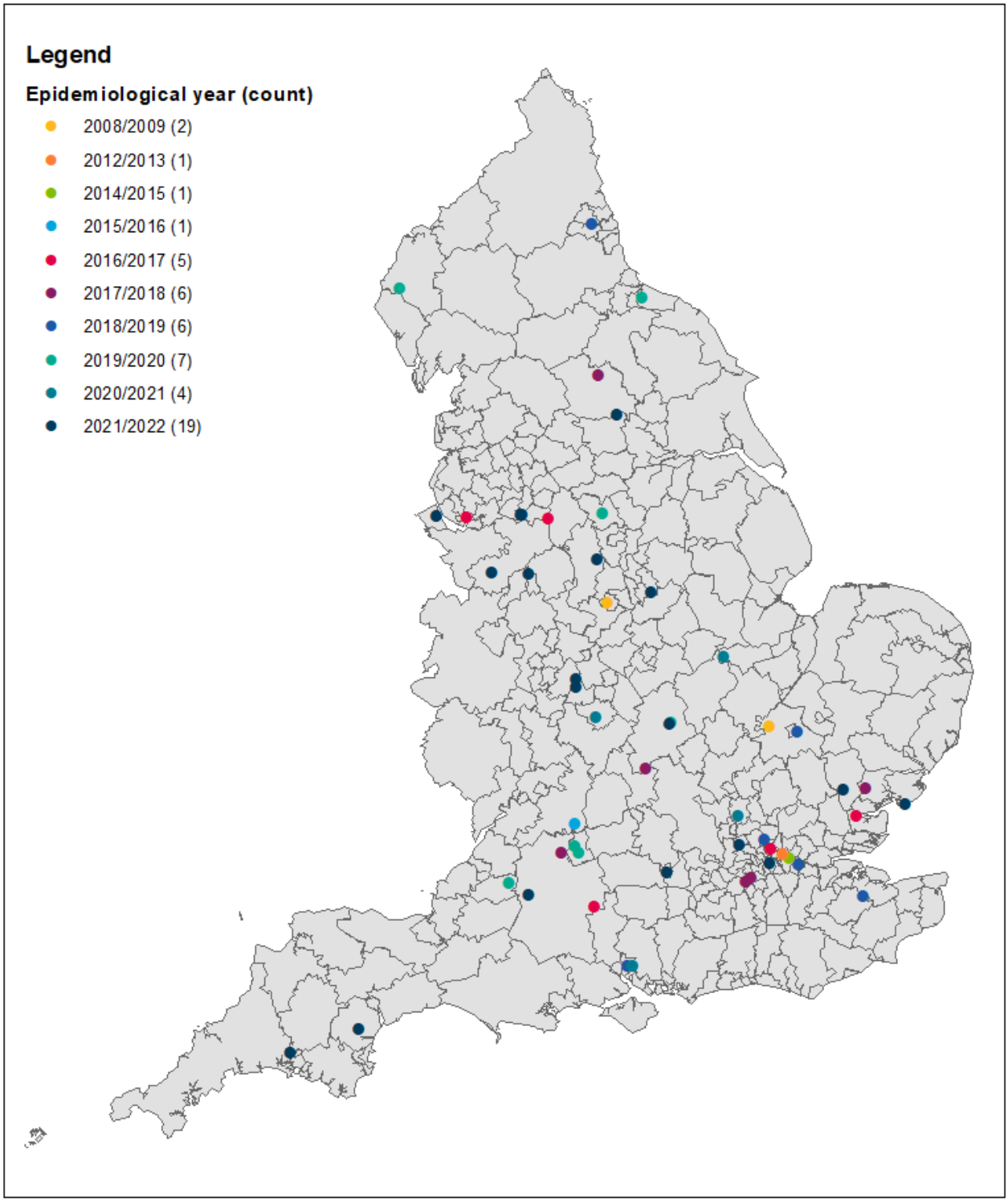


Figure B. Distribution of MLST profiles among UK Hia and Hib collection. ST frequency across the 102-isolate collection of Hia and Hib.

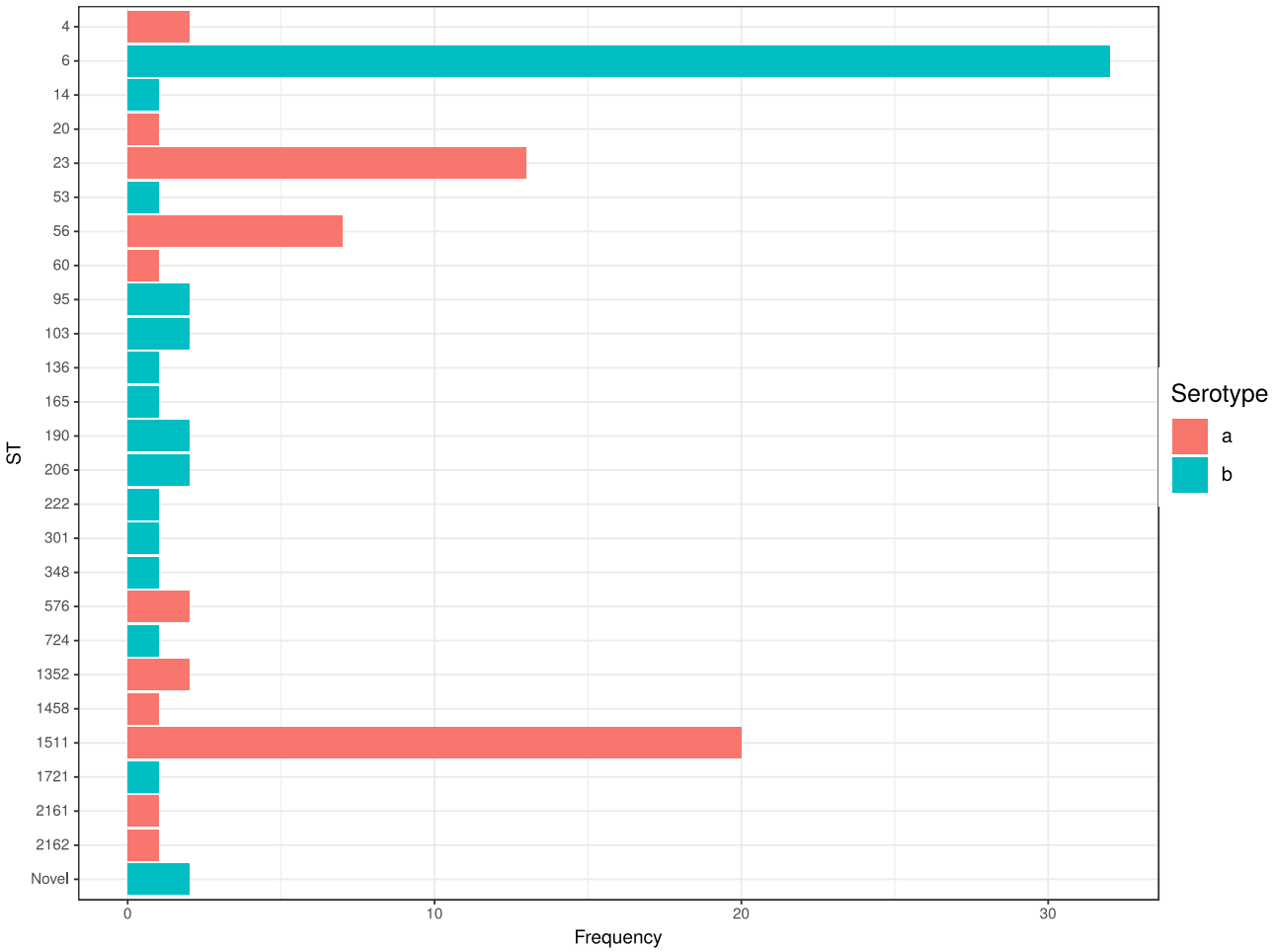
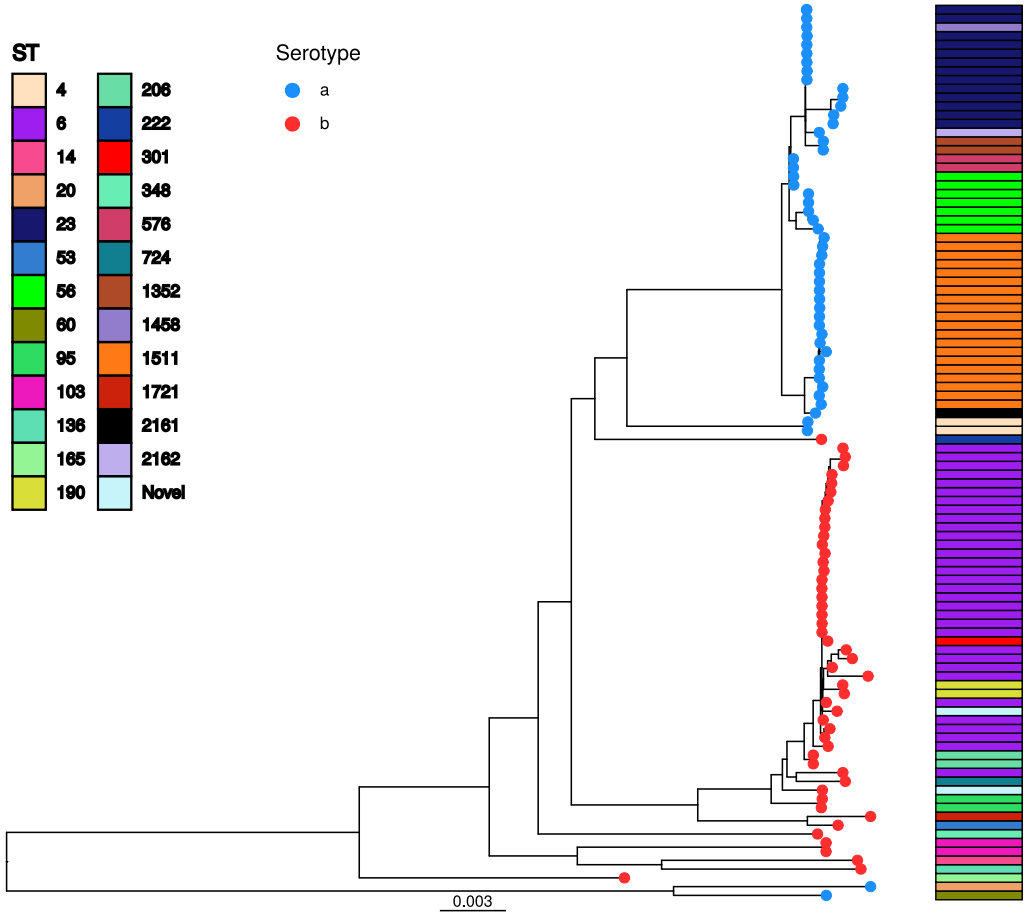


Figure C. Core-genome SNP phylogeny and MLST of 51 Hia and 51 Hib isolates from England.

Tree drawn using a maximum-likelihood implemented in IQ-Tree. Tips of the phylogeny are colored by isolate serotype. Vertical bars represent the ST of the corresponding tip in the phylogeny.



STROBE Statement: checklist

	Item No	Recommendation	Section (Paragraph)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**Title and abstract**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**Title and abstract**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**Introduction (P2)**
Objectives	3	State specific objectives, including any prespecified hypotheses	**Introduction (P2)**
Methods			
Study design	4	Present key elements of study design early in the paper	Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods (P1, data analysis, WGS)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Observational national surveillance, methods describe case definition and follow-up methods
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods
Bias	9	Describe any efforts to address potential sources of bias	Methods – adjustment for missing serotypes
Study size	10	Explain how the study size was arrived at	N/A
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Methods – data analyses
		(*b*) Describe any methods used to examine subgroups and interactions	Methods
		(*c*) Explain how missing data were addressed	Methods
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Methods and results
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	Not included
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*Results&Tables*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results
		(*b*) Report category boundaries when continuous variables were categorized	Results
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion P1
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion – P11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Conclusion

Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding source

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