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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Summary

Background Invasive *Haemophilus influenzae* serotype a (Hia) disease is rare, with most cases reported among Indigenous populations in North America. In England, national surveillance was enhanced following an increase in laboratory-confirmed invasive Hia disease since the 2016–17 epidemiological year. This study aimed to describe the epidemiological trends, clinical characteristics of cases, and assess potential genomic drivers.

Methods Hospital laboratories in England routinely submit invasive *H influenzae* isolates to the UK Health Security Agency for confirmation and serotyping. In this prospective national surveillance study we contacted the general practitioners and clinicians of all patients with laboratory-confirmed invasive Hia from the 2008–09 to the 2021–22 epidemiological year to complete a clinical questionnaire on demographics, underlying conditions, clinical presentation, complications, outcomes, and travel history of the patient. All Hia invasive isolates from residents in England were included in the study; non-invasive isolates were excluded. Multilocus sequence typing (MLST), whole genome single-nucleotide polymorphism, and k-mer-based analysis of bacterial isolates were performed following Illumina whole-genome sequencing (WGS). Outcomes included epidemiological trends, clinical characteristics of confirmed Hia cases, and genomic analyses.

Findings From the 2008–09 to the 2021–22 epidemiological years, there were 52 cases of invasive infection with *H influenzae* serotype a in England (25 [48%] in female patients and 27 [52%] in male patients). There were zero to two annual Hia cases (accounting for <0·5% of serotyped *H influenzae* isolates) until 2015–16, after which cases increased across England to 19 cases in 2021–22 (incidence 0·03 cases per 100 000), when Hia accounted for 19 (4%) of 484 serotyped *H influenzae* isolates, 19 (19%) of 100 capsulated cases, and 37% (19 of 52) of all *H influenzae* cases between 2008–09 and 2021–22. Most of the recent increase in cases occurred among individuals aged 65 years and older (17 [33%] of 52), who typically presented with bacteraemic pneumonia (13 [76%] of 17), and infants younger than 1 year, who had the highest incidence and were more likely to present with meningitis (five [50%] of ten). Overall case fatality rate was 7·7% (95% CI 2·1–19·7; four of 52 patients). WGS found that closely related MLST sequence types ST1511 (20 [39%] of 51), ST23 (13 [25%] of 51), and ST56 (seven [14%] of 51) accounted for most cases, with no evidence of serotype b strains switching capsule to Hia. Duplication of the capsule operon, associated with more severe disease, was present in 32 (80%) of 40 of these sequence types. Analysis of the core and accessory genome content grouped most isolates into a single strain.

Interpretation The persistent increase in invasive Hia cases across England and across all age groups suggests widespread transmission, consistent with reports from other European countries, and will require close monitoring.

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Introduction

Haemophilus influenzae is a Gram-negative coccobacillus commonly carried in the human nasopharynx, which can cause non-invasive upper respiratory and ear infections, such as sinusitis and otitis media, as well as serious invasive disease, including septicaemia and meningitis.[1] *H influenzae* can be distinguished into six serotypes based on their unique polysaccharide capsule (a–f), and non-encapsulated (or non-typeable *H influenzae* [NTHi]) strains. *H influenzae* serotype b (Hib) was historically the most prevalent serotype causing invasive disease, but is now rare because of the successful implementation of the Hib conjugate vaccines into national childhood immunisation programme in most countries,[1,2] including England.[3] Consequently, NTHi became the most common cause of invasive *H influenzae* cases, with very few cases due to encapsulated *H influenzae*, mainly serotype f followed by serotype e.[1,2,4] Until around 10 years ago, invasive disease due to *H influenzae* serotype a (Hia) was extremely rare, except among Indigenous populations in North America and Australia.[5,6] In such populations, the clinical manifestations of invasive Hia disease were similar to Hib, typically causing meningitis and

Research in context

Evidence before this study

We searched PubMed using the terms ((“haemophilus influenzae serotype a”[Title/Abstract]) OR (“h. influenzae serotype a”)) OR ((hia[Title/Abstract]) AND (influenzae[Title/Abstract])) in Dec 31, 2022, with no date restrictions, and used the snowball process to identify additional relevant publications. Only English publications were included. We excluded publications unrelated to *Haemophilus influenzae*. Invasive *H influenzae* disease has declined because of routine immunisation against *H influenzae* serotype b (Hib) globally. Most invasive *H influenzae* cases are due to non-typeable strains, followed by *H influenzae* serotype f in most countries. Invasive *H influenzae* caused by serotype a (Hia) is rare worldwide, except among indigenous populations in Canada and Alaska, where the epidemiology is similar to that of Hib, causing severe disease in the extremes of age, typically meningitis in young children and pneumonia in adults with underlying conditions. Elsewhere, invasive Hia cases were rare until recently and, although case numbers remain very low, several European countries have observed an increase in cases. Speculated reasons for the recent increase include serotype replacement disease, capsular switching from the more virulent Hib to Hia, or increased virulence potentially caused by duplication of the capsule operon. Because invasive Hia disease is so rare outside populations at high risk, there are limited data on epidemiological trends, genomic characterisation of invasive isolates, clinical features, or outcomes, especially when related to the recent increase in cases.

Added value of this study

We have presented the most comprehensive analysis of the largest cohort with invasive Hia disease in Europe, including both epidemiological, clinical, and genomic assessment of the recent increase in cases affecting all age groups, which was identified through a well established national surveillance programme spanning more than three decades in England. A small increase in invasive Hia cases, first observed in the 2016–17 epidemiological year (July until June the following year), has persisted, with the largest case numbers observed during 2021–22, despite a small decline, mainly in older adults, due to pandemic restrictions in 2020–21 compared with the previous year. This increase has been observed across the country, affecting all age groups, indicating widespread transmission across England. Detailed genomic analysis found that most strains (47 [92%] of 51) belong to the same clade within a global phylogeny, with no evidence of capsule-switching from Hib strains, with multilocus sequence typing sequence types ST1511 (n=20), ST23 (n=13), and ST56 (n=7) accounting for most invasive cases (40 [78%] of 51).

Implications of all the available evidence

The recent increases in the past decade in invasive Hia disease has affected many European countries and, in England, has persisted for more than 5 years, with cases continuing to increase after pandemic restrictions were lifted in England. Hia typically causes meningitis in infants and pneumonia in older children and adults, with an overall case-fatality rate of 7·7% (four of 52). Current evidence indicates that Hia will continue to cause invasive cases and will require global close monitoring in the coming years.

septicaemia in children younger than 2 years, and adults with invasive Hia disease usually had underlying comorbidities and presented with pneumonia, septic arthritis, and osteomyelitis.[1]

In England, where invasive *H influenzae* is a notifiable disease, national surveillance identified an increase of invasive Hia disease during the 2016–17 epidemiological year (from July 1, 2016, to June 30, 2017), mainly among adults aged 45 years or older. We, therefore, enhanced our national surveillance to collect additional clinical and epidemiological data for confirmed cases, along with detailed characterisation, including whole-genome sequencing (WGS), of invasive Hia isolates. Here, we report the findings of our investigations over a 14-year period, which included the COVID-19 pandemic with national restrictions and lockdowns.

See Online for appendix

Methods

Surveillance methods

The UK Health Security Agency (UKHSA; formerly Public Health England) has been conducting national surveillance in England for more than three decades and provides a national service for confirmation and serotyping of invasive *H influenzae*.[3] UKHSA receives electronic laboratory reports of clinically significant pathogens from all National Health Service (NHS) hospital laboratories through the Second Generation Surveillance System (appendix p 2) and routinely contacts the laboratories to submit invasive isolates for confirmation and serotyping to its national reference laboratory if not already done.[3] Our surveillance does not include PCR positive only samples. However, hospitals in England rarely perform PCR testing for *H influenzae* other than occasionally for cerebrospinal fluid (CSF) samples. If positive, the hospital will link with the UKHSA national reference laboratory. Further details on confirmation and serotyping of bacterial isolates are described in the appendix (p 2). All Hia invasive isolates from residents in England were included in the study. Specimens received and confirmed to be from residents outside of England (eg, Scotland) were excluded.

Invasive *H influenzae* disease, was defined as isolation of the pathogen from a normally sterile site. Meningitis was defined as *H influenzae* from CSF or from blood with clinical or radiological evidence of meningitis. Classification of other clinical presentations (bacteraemic pneumonia, septic arthritis, osteomyelitis, epiglottitis, and cellulitis) was based on isolation of *H influenzae* from a normally sterile site with symptoms and signs consistent with that clinical presentation. Septicaemia was defined as *H influenzae* isolated from the blood with no focal clinical signs.

UKHSA has legal permission to process confidential information for national surveillance of communicable diseases without individual patient consent (Regulation 3 of Health Service Regulations 2002) and, as such, ethics committee approval is not required.

Genomic analyses

WGS, assembly, and MLST typing of Hia isolates was performed as described in the appendix (p 2). To test for possible serotype switching from serotype b (Hib) to serotype a, a panel of 52 Hib isolates, collected from 2006 to 2021 from routine surveillance in England, were also analysed. WGS and assembly of these Hib isolates was performed as described in the appendix (p 2), with one isolate failing quality control, leaving 51 Hib isolates. Snippy (version 4.6.0) was used to create a core-genome alignment of the Hia and Hib isolates, mapping isolates to the 10810 Hib isolate (NCBI accession code NC_016809). Phylogenetic analysis was then performed with IQ-Tree (version 2.0.3) on the core-genome alignment.[7]

To determine how the UKHSA Hia sequences are related to *H influenzae* isolates globally, all available *H influenzae* assemblies were downloaded from the National Center for Biotechnology Information (NCBI) RefSeq database on Nov 28, 2022. Contigs of less than 500 bp were removed from the assemblies and QUAST[8] (version 5.2.0) was used for quality control. PopPUNK (version 2.5.0) was then used to delineate the combined RefSeq (n=841) and English Hia assemblies (n=51), a total of 892 assemblies, into unique strains based on core and accessory genome distances.[9] From this fit, five RefSeq assemblies were removed due to extreme accessory divergence, leaving 836 RefSeq assemblies. PopPUNK was then used to create a core-genome distance phylogeny for the final collection of 887 sequences.

To assess the extent of capsule locus duplication in the Hia collection, typically associated with *IS1016-bexA* partial deletion,[10] an approach similar to that by Topaz and colleagues was used.[11] Namely, reads were mapped against the assemblies produced above for each isolate, with the mean depth of coverage across the genome calculated. The hicap tool (version 1.0.3) was then used to extract the capsule locus from each isolate and reads were mapped against this locus.[12] The ratio of the depth of coverage for the overall assembly and the capsule locus was then determined. MLST profiles were used to classify isolates into four genetic groupings, based on allelic distances between clusters, to compare bacterial groupings with epidemiological and clinical parameters.

Data analyses

Laboratory-confirmed invasive Hia cases during 2008–09 to 2021–22 were then followed up by requesting the patient's general practitioner to complete a questionnaire on additional demographics, underlying conditions, clinical presentation, complications, outcomes, and travel history. Date of birth and sex information (categorised as male or female) were obtained from the Patient Demographic Service, an electronic database of all patients regstered in the NHS. Questionnaires for Hia cases confirmed before 2016–17 (when enhanced surveillance was initiated) were completed retrospectively by the general practitioner, and questionnaires for subsequent cases were completed prospectively. When needed, hospital clinicians were contacted for any missing information in the questionnaire. Fatalities were additionally identified through linkage with the Patient Demographic Service and electronic death registration records provided by the Office for National Statistics (ONS) for public health surveillance. Clinical presentation was categorised hierarchically as meningitis, pneumonia, another focus, and septicaemia. Case fatality rates (CFR) are reported as deaths within 30 days of the specimen collection date, with 95% CIs calculated using the Poisson binomial method. We confirmed the cause of death with data from the death certificate provided by the general practitioner or extracted from ONS. Data were analysed using Stata (version 17.0). Continuous variables that did not follow a normal distribution were described as median and IQR. Categorical variables were expressed as proportions and compared using the χ^2 test, or Fisher exact test as appropriate. Serotype-specific incidence was calculated using adjusted case numbers (assuming serotype distribution among isolates that were not serotyped was the same as in serotyped isolates by multiplying by a correction factor equal to total number of *H influenzae* cases divided by number of serotyped *H influenzae* cases) and mid-year ONS population estimates for England.

For more in **Snippy** see https://github.com/tseemann/snippy

Role of the funding source

Surveillance design, data collection, data analysis, data interpretation, and writing of the report was performed by employees of the funder, UKHSA.

Results

During the 14-year surveillance period (2008–09 to the 2021–22 epidemiological years), there were zero to two invasive Hia cases per year until 2016–17 when case numbers increased, with 42 (81%) of 52 cases occurring in the five most recent epidemiological years (2017–18 to

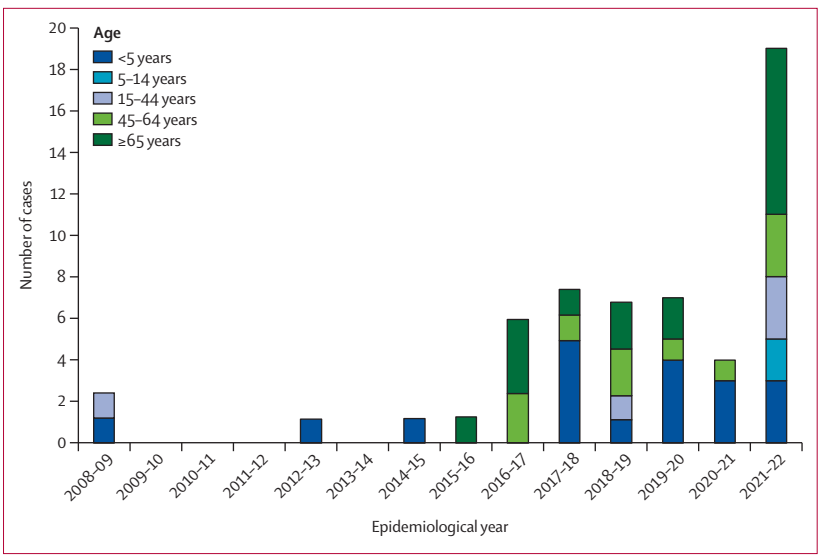


Figure 1: **Number of *Haemophilus influenzae* serotype a cases by age group and epidemiological year, 2008–09 to 2021–22 in England**

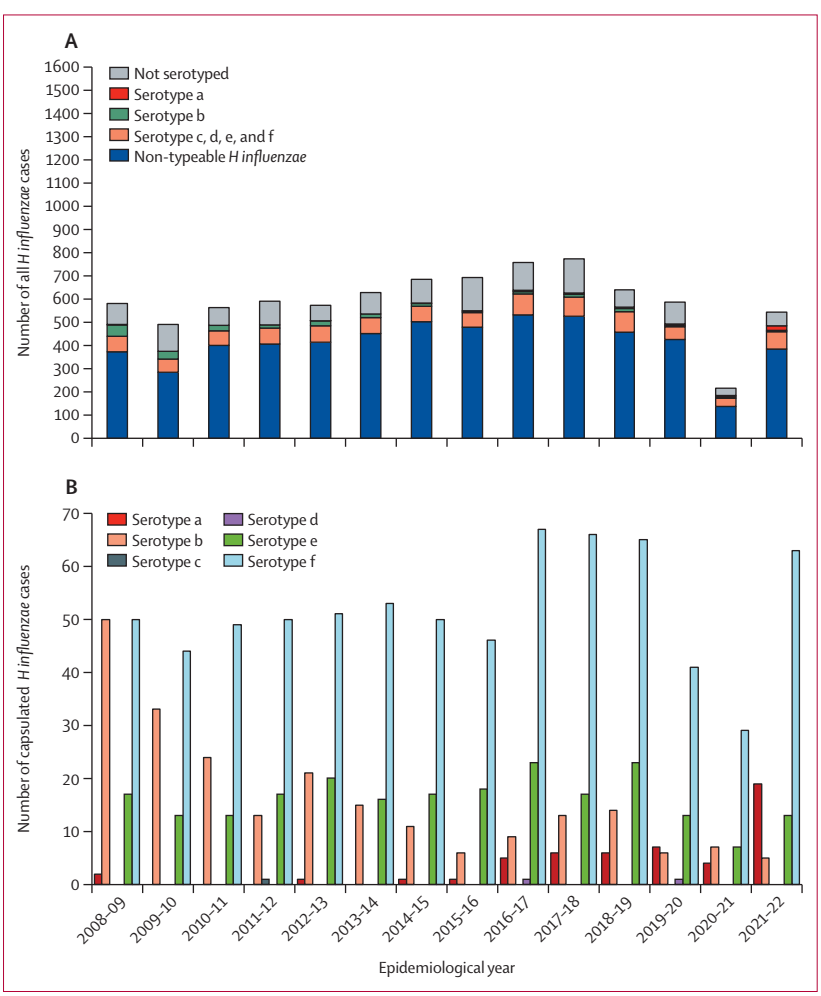


Figure 2: **Number of *Haemophilus influenzae* cases by epidemiological year and serotype (A), with details for only encapsulated *H influenzae* cases by epidemiological year (B), 2008–09 to 2021–22 in England**

2021–22) and 19 (37%) of 52 cases during 2021–22 (figure 1). Hia, however, remains a rare cause of invasive *H influenzae* disease (figure 2). 7002 (84·2%) of 8316 *H influenzae* isolates were serotyped during the 14-year period. Of those, 5769 (82·4%) were confirmed as NTHi, 1181 (16·9%) as other capsulated serotypes (b, c, d, e, and f), including 227 (3·2%) as Hib, and 52 (0·7%) as Hia (figure 2).

During the first COVID-19 pandemic year (2020–21), invasive Hia cases declined compared with the previous 3 years, in keeping with total invasive *H influenzae* cases, but increased rapidly the following year, such that invasive Hia case numbers were highest during 2021–22 (n=19), accounting for 19 (4%) of the 484 cases with serotyped isolates (484 [89%] of 543) and 19 (19%) of the 100 cases due to encapsulated *H influenzae* (figure 1; figure 2). The increase in invasive Hia cases since 2016–17 was first noted in adults aged 65 years and older and younger adults (aged 45–64 years), followed by children younger than 5 years (figure 1), who had the highest incidence in 2021–22. In 2021–22, annual invasive Hia disease incidence per 100 000 was 0·33 for patients younger than 1 year (n=2), 0·04 for patients aged 1–4 years (n=1), 0·03 for patients aged 5–14 years (n=2), 0·01 for patients aged 15–44 years (n=3), 0·02 for patients aged 45–64 years (n=3), and 0·08 for patients aged 65 years and older (n=9), with an overall annual incidence of 0·03 per 100 000 (n=19). The overall adjusted incidence, after accounting for non-serotyped isolates (59 [11%] of 543), was 0·04 per 100 000 (n=21). This adjustment did not affect the age-group-specific incidence due to small case numbers. For comparison, invasive Hib disease incidence during 2021–22 was 0·00 per 100 000 for patients younger than 15 years (n=0), 0·02 for patients aged 45–64 years (n=3), and 0·01 for patients aged 65 years and older (n=1), with an overall annual incidence of 0·009 per 100 000.

The median age of patients with Hia was 55 years (IQR 1–67). 44 (86%) of 51 patients with Hia with a known ethnic group were White (table 1). We did not identify any temporal or geographical associations between the cases since 2016–17 (appendix p 3). Of 31 patients with known recent travel history, two had recently travelled to the USA, one during 2018–19 and the other during 2019–20.

Completed questionnaires were returned for all Hia cases. The prevalence of underlying conditions known to increase the risk of invasive *H influenzae* disease increased with age, from 10% (one of ten) of patients younger than 1 year to 76% (13 of 17) of patients aged 65 years and older (table 1). The most common clinical presentation was pneumonia, especially among patients aged 65 years and older, whereas in infants, meningitis was the most common clinical presentation (five [50%] of ten; table 1). Overall, clinical presentation was similar in previously healthy individuals compared with those with comorbidities, except in patients younger than

	Age <1 year (n=10)	Age 1–4 years (n=8)	Age 5–14 years (n=2)	Age 15–44 years (n=5)	Age 45–64 years (n=10)	Age ≥65 years (n=17)	Total (n=52)
Sex							
Female	2 (20%)	2 (25%)	1 (50%)	2 (40%)	7 (70%)	11 (65%)	25 (48%)
Male	8 (80%)	6 (75%)	1 (50%)	3 (60%)	3 (30%)	6 (35%)	27 (52%)
Ethnicity							
White	7 (70%)	7 (100%)*	1 (50%)	4 (80%)	10 (100%)	15 (88%)	44 (86%)*
Non-White	3 (30%)	0*	1 (50%)	1 (20%)	0	2 (12%)	7 (14%)*
Comorbidities							
Any comorbidity†	1 (10%)	4 (50%)	1 (50%)	3 (60%)	6 (60%)	13 (76%)	28 (54%)
Chronic lung disease	0	2 (50%)	1 (100%)	0	3 (50%)	10 (77%)	16 (57%)
Chronic liver or gastrointestinal disease	0	0	0	1 (33%)	1 (17%)	0	2 (7%)
Chronic heart disease	1 (100%)	1 (25%)	0	1 (33%)	1 (17%)	3 (23%)	7 (25%)
Chronic renal disease	0	0	0	0	1 (17%)	1 (8%)	2 (7%)
Metabolic disease	0	0	0	0	0	3 (23%)	3 (11%)
Malignancy or immunosuppression	0	1 (25%)	0	0	1 (17%)	5 (38%)	7 (25%)
Chromosomal abnormalities	1 (100%)	2 (50%)	1 (100%)	0	0	0	4 (14%)
Main clinical presentation							
Meningitis	5 (50%)	1 (13%)	0	0	1 (10%)	0	7 (13%)
Pneumonia	2 (20%)	4 (50%)	1 (50%)	3 (60%)	4 (40%)	13 (76%)	27 (52%)
Other focus‡	3 (30%)	0	1 (50%)	1 (20%)	3 (30%)	1 (6%)	9 (17%)
Septicaemia	0	3 (38%)	0	1 (20%)	2 (20%)	3 (18%)	9 (17%)
Time period							
2008–09 to 2012–13	2 (20%)	0	0	1 (20%)	0	0	3 (6%)
2013–14 to 2017–18	3 (30%)	2 (25%)	0	0	3 (30%)	5 (29)	13 (25%)
2018–19 to 2021–22	5 (50%)	6 (75%)	2 (100%)	4 (80%)	7 (70%)	12 (71%)	36 (69%)
30-day fatality	1 (10%)	0	0	0	0	3 (18%)	4 (8%)

Data are n (%). *Missing one data point in the 1–4 years age group. †The total of cases with any comorbidity might not add up to the number of cases by individual comorbidities as cases might have more than one condition. ‡Other foci of infection included epiglottitis (n=4), septic arthritis (n=3), and cellulitis (n=2).

***Table 1: Haemophilus influenzae* serotype a cases by demographics and clinical information, 2008–09 to 2021–22 in England**

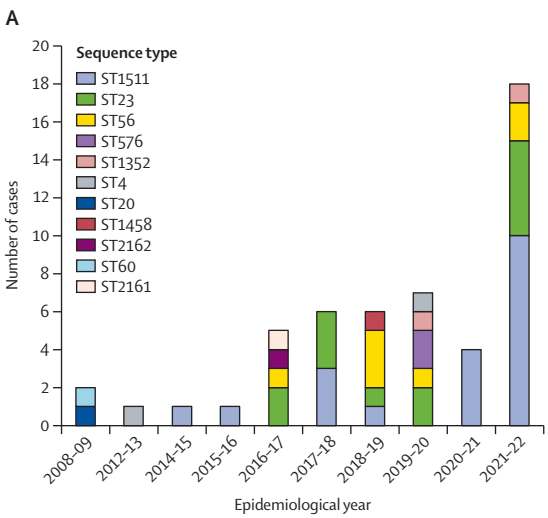
15 years, in whom meningitis was more frequent in previously healthy children as was septicaemia in those with underlying conditions (five [36%] of 14 and three [50%] of six; Fisher's exact test, p=0·038).

Of the 52 patients with confirmed invasive Hia, four died within 30 days (30 day CFR 7·7% [95% CI 2·1–19·7]): an infant (aged <1 year) with multiple comorbidities who presented with meningitis and three older adults (>65 years) who had bacteraemic pneumonia. All four deaths were attributed to Hia. Of these deaths in adults, two had cardiac and respiratory comorbidities and one was reported to be previously healthy. Three cases were due to ST1511 strains and one to a ST2162 strain.

Bacterial isolates from 51 (98%) of 52 Hia infections were available for genomic analysis. MLST analysis revealed that most cases were caused by ST1511 (20 [39%] of 51), ST23 (13 [25%] of 51), and ST56 (seven [14%] of 51); ST1511 was first seen in 2014–15, and ST23 and ST56 were identified in 2016–17 (figure 3A). These three sequence types are closely related, being only single or double locus variants of each other or most of the other sequence types (figure 3B). Only three sequence types (n=4) were not related to the main cluster: ST4, ST20, and ST60.

For comparison, isolates were categorised into four groups, according to their frequency and relatedness: group 1 (ST1511), group 2 (ST23), group 3 (ST2161, ST56, ST2162, ST1458, ST576, and ST1352), and group 4 (ST4, ST60, and ST20; figure 3B). From 2008–09 to 2012–13, only group 4 isolates were identified, with only one ST4 observed in 2020. From 2018–19 to 2021–22, 15 (43%) of 35 isolates were due to ST1511 alone, compared with five (38%) of 13 isolates from 2013–14 to 2017–18, and none from 2008–19 to 2012–13. Numbers were too small to assess evidence of significant associations between epidemiological and genomic data (table 2).

Comparison of the Hia genomes with a global *H influenzae* genome dataset using PopPUNK revealed that all the English Hia isolates, except for the highly divergent group 4 isolates (ST4, ST60, and ST20), were located within a single strain comprising 64 global Hia isolates, annotated as Strain 1, which also included Hia isolates from international sources including Canada, USA, Japan, Brazil, Portugal, and Spain (figure 4). A core-genome distance phylogeny revealed that Strain 1 comprised a monophyletic clade. Hence, the UK isolates were very closely related to each other and to the



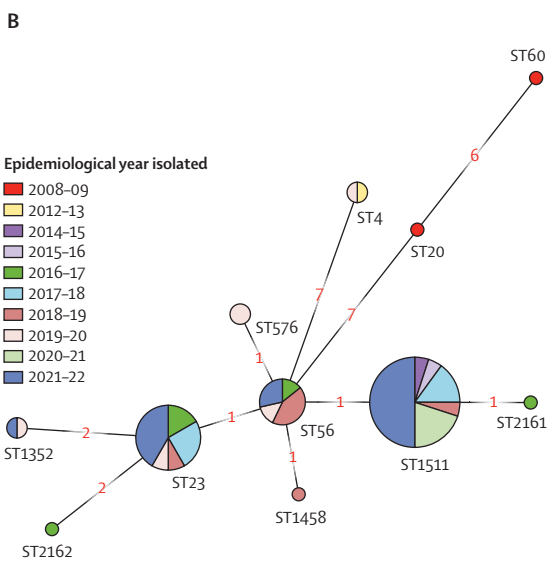


Figure 3: **Frequency of each sequence type by epidemiological year (A) and minimum spanning tree showing relatedness of each sequence type (B)**
In B, the number of the seven loci with allelic differences between each sequence type is depicted using a number.

	Group 1* (n=20)	Group 2† (n=13)	Group 3‡ (n=14)	Group 4§ (n=4)
Clinical presentation				
Meningitis	3 (15%)	1 (8%)	2 (14%)	1 (25%)
Pneumonia	12 (60%)	6 (46%)	7 (50%)	1 (25%)
Other	2 (10%)	3 (23%)	3 (21%)	1 (25%)
Septicaemia	3 (15%)	3 (23%)	2 (14%)	1 (25%)
Age group				
<1 year	5 (25%)	3 (23%)	0	2 (50%)
1–4 years	2 (10%)	4 (31%)	2 (14%)	0
5–14 years	0	1 (8%)	1 (7%)	0
15–44 years	1 (5%)	2 (15%)	1 (7%)	1 (25%)
45–64 years	5 (25%)	2 (15%)	3 (21%)	0
≥65 years	7 (35%)	1 (8%)	7 (50%)	1 (25%)
Epidemiological year group				
2008–09 to 2012–13	0	0	0	3 (75%)
2013–14 to 2017–18	5 (25%)	5 (38%)	3 (21%)	0
2018–19 to 2021–22	15 (75%)	8 (62%)	11 (79%)	1 (25%)

Data are n (%). MLST groups were grouped based on frequency and relatedness. MLST=multilocus sequence typing. *ST1511. †ST23. ‡ST2161, ST56, ST2162, ST1458, ST576, and ST1352. §ST4, ST60, and ST20.

Table 2: ***Haemophilus influenzae*** **cases by bacterial MLST grouping, clinical presentation, age group, and time period**

international isolates despite some diversification into individual MLSTs.

When comparing Hia and Hib isolates from England, there was no overlap between MLSTs (appendix p 4). An alternative core-genome single-nucleotide polymorphism (SNP)-based phylogeny also split most Hia isolates into a monophyletic grouping separate from Hib isolates (appendix p 5). The outlier Hia strains were also unrelated to the Hib strains. Hence, we found no evidence of serotype switching between Hib and Hia within the English *H influenzae* population.

Analysis of WGS raw data from the English Hia isolates, to test for possible capsule duplication among Hia, showed that 36 (72%) of 50 isolates had a depth of coverage of more than 1·5× for their capsule region compared with their overall genome, which indicates a probable duplication of the region. Of the three commonest sequence types, 12 (92%) of 13 ST23, 14 (70%) of 20 ST1511, and six (86%) of seven ST56 isolates had this probable capsule duplication.

Discussion

England is having a small but steady increase in invasive Hia disease, which was previously exceptionally rare in most parts of the world. Before the recent increase (2016–17), sporadic cases occurred mainly in young children and were due to different MLST strains but, since 2016–17, cases increased across all age groups, mainly due to expansion of a single strain. COVID-19 pandemic restrictions were associated with a small temporary decline in all invasive *H influenzae* cases during 2020–21,[13] including Hia in adults. However, cases increased rapidly the following year, such that serotype a was responsible for 19 (4%) of the 484 invasive *H influenzae* disease cases and 19 (19%) of 100 invasive disease cases due to encapsulated *H influenzae*. Pneumonia was the main clinical presentation, except in infants who were more likely to present with meningitis. Comorbidity prevalence increased with age and was reported in more than half the older adults. Most patients recovered without sequelae, but four died of their infection, giving an overall CFR of 7·7% (four of 52).

Before the recent increase, Hia was very rare, except in specific populations at high risk, namely the Indigenous

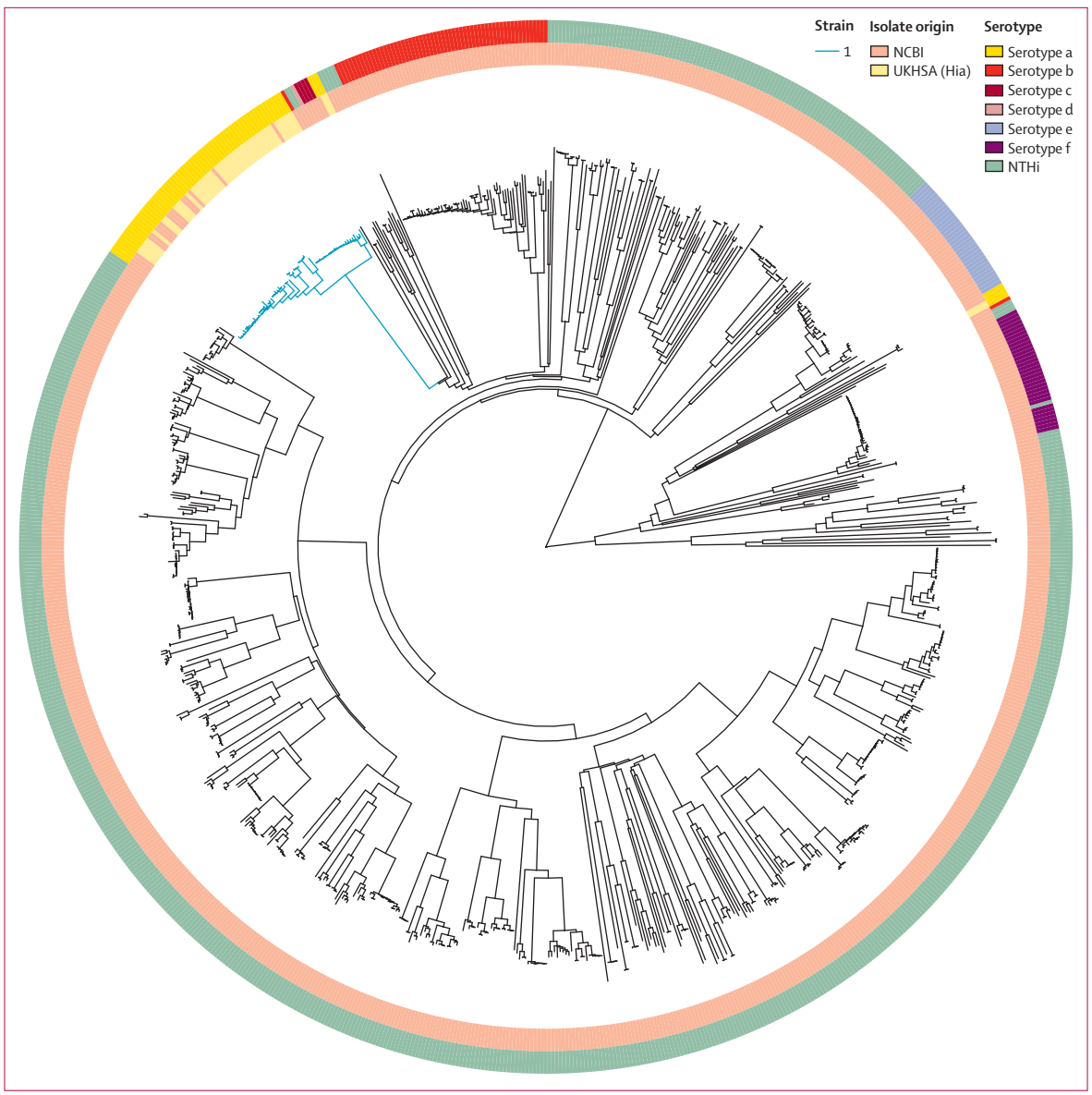


Figure 4: **Core-genome phylogeny of 887 *Haemophilus influenzae* isolates**
Phylogeny was formed from the core-genome distances estimated by PopPUNK on a collection of 836 NCBI RefSeq assemblies and 51 English Hia isolates. The strain 1 clade is highlighted in blue. The inner ring represents the source of an isolate (either NCBI RefSeq or the UKHSA Hia collection) and the outer ring represents the capsule type of the isolate. Hia=*H influenzae* serotype a. NCBI=National Center for Biotechnology Information. NTHi=non typeable *H influenzae* (non-capsulated). UKHSA=UK Health Security Agency.

populations in North America and Australia—the same populations that were also at increased risk of Hib disease before routine immunisation.[1] In these populations, the epidemiology was similar to Hib, with the highest incidence reported in infants who typically presented with meningitis.[6,14]

In the USA, Hia cases have also been reported to have been gradually increasing nationally from 2008 to 2017, not only among Alaska Natives and American Indians,[14,15] with an annual average incidence of 0·10 cases per 100 000 compared with 0·03 per 100 000 in our cohort during 2021–22; in infants (aged <1 year), who had the highest incidence, with 1·6 cases per 100 000 in the USA[14] and 0·33 cases per 100 000 in our cohort. In Canada, and other areas with Indigenous populations, Hia is the most prevalent serotype[5,15,16] with a higher incidence among children younger than 5 years (7·5–50·0 cases per 100 000).[1,5,17] The reason for such a difference in the incidence of Hia in England compared with North American countries is unclear but might be determined by population differences as well as genomic differences of Hia isolates.

A recent emergence or increase in invasive Hia cases has also been reported in Europe, including in Italy, Spain, Ireland, and Portugal.[1,18–21] In Norway, Hia cases increased from 2018 to 2021 (from one to seven), accounting for 15 (22%) of 69 invasive cases due to encapsulated *H influenzae* during this period.[22] Notably, Hia cases did not decline during pandemic restrictions in 2020, despite an overall decrease in *H influenzae* cases, and increased in 2021.[22] In France, Hia cases also increased from 2017 to 2021, affecting mostly children younger than 5 years (30 [68%] of 44 cases).[23] Interestingly, Hia cases peaked in 2020 and were unaffected by pandemic restrictions.[23] In Argentina, the proportion of Hia cases increased from 12% (11 of 93) to 15% (22 of 150) of all *H influenzae* cases from 2011–19, with 54% of cases occurring in childeren younger than 1 year.[24] Compared with other countries, however, Hia accounts for a very small proportion of invasive serotyped *H influenzae* cases in England (19 [4%] of 484).

The most common presentation of invasive Hia disease is pneumonia, which typically affects adults with underlying comorbidities, and meningitis was the most common presentation in patients younger than 5 years, especially infants with no known comorbidities.[5,14,15,25] Before routine immunisation, a similar pattern was also observed with Hib.[1,4] Our CFR of 7·7% is similar to the US national CFR (7·8%),[14] and lower than North American Arctic populations (10%).[5]

Invasive Hia disease represents the most severe end of the clinical spectrum of infection. As with other *H influenzae*, Hia can also cause non-invasive respiratory and ear infections, including sinusitis and otitis media.[1] Although *H influenzae* is commonly carried in the nasopharynx, with reported prevalence of 14–87%, it is mostly predominated by NTHi (96–100%),[26–28] with Hia carriage prevalence of 0·0–0·3%.[26–28] Interestingly, an Alaskan study following a Hia cluster found that close contacts of patients had higher Hia carriage rates than non-contacts (14·8% *vs* 1·9%), and the rate among close contacts reduced to be similar to that of non-contacts following antibiotics, indicating that antibiotic prophylaxis in close contacts could interrupt Hia transmission and help prevent secondary cases in populations at high risk.[29] Because Hib conjugate vaccines provide serotype-specific protection by inducing immunity against the unique Hib polysaccharide capsule, they will not offer any cross-protection against other *H influenzae* serotypes or NTHi. An Hia conjugate vaccine is in development, similar to the Hib conjugate vaccine, which will not only protect against Hia disease but also prevent carriage acquisition and, potentially, provide population protection (herd immunity) if the immunisation programme can be targeted towards the primary carriage group.[30] In England, as in Europe, recent cases were identified across the country and across all age groups, indicating that carriage might be now more widespread (appendix p 3).

MLST showed that ST1511, ST23, and ST56 were responsible for most invasive Hia cases in England, with most of the remaining being closely related single or double locus variants of these sequence types. Only four isolates had the divergent sequence types ST4, ST20, or ST60. All these sequence types have been previously described as causing invasive Hia disease. ST23 is among the most common sequence types in North America,[5,16] but was only reported in Europe after 2015, including Italy, Spain, and Norway.[18,20,22] In England, ST23 was first identified in 2016 and was, overall, the second most prevalent sequence type, accounting for 25% (13 of 51) of isolates from 2008–09 to 2021–22. It is, therefore, probable that ST23 was imported into Europe from North America.

A 2022 international genomic analysis of Hia isolates concluded that Hia formed four major clades by SNP analysis, and most Hia isolates belonged to clade 1, predominated by ST56, ST23, and ST576. This clade was more closely related to Hib, Hid, and Hic.[11] ST1511, the most common sequence type in our study, and ST56, the third most common type, are both closely related single locus variants of ST23.[11] ST1511 appears to have emerged recently and was first assigned in the international MLST database to an Australian Hia blood isolate from 2014.[31] Despite differences in MLST type, our whole genome k-mer analysis within a global *H influenzae* context using PopPUNK categorised almost all strains from England within a monophyletic clade that was part of a single global strain. Hence, the recent isolates from England are very closely related despite differences in MLST types and can be seen as largely clonal.

Interestingly, the international genomic analysis found that most clade 1 isolates had a likely capsule duplication, which has been associated with increased disease severity.[10,11] We performed a similar analysis and also found that most of the English ST1511, ST23, and ST56 strains also contain the capsule duplication.

Another potential mechanism for increased Hia virulence is capsule switching from Hib (polyribose ribitol phosphate linked by phosphodiester bonds) to Hia (glucose and ribitol polymers linked by phosphodiester linkages).[1,32] We did not identify an overlap between the Hia MLST types and recent invasive Hib strains in England. In-depth SNP comparison of their genomes also did not find any evidence of capsule switching among Hia strains.

The well established national surveillance system in England provides a reliable source of epidemiological and laboratory surveillance data, with a consistently high proportion of invasive isolates serotyped. This surveillance allowed us to detect very small increases in cases as soon as they emerged in 2008–09. The detailed follow-up with 100% questionnaire completion rates was also crucial for understanding disease characteristics and outcomes of such rare infections. Access to a single national reference laboratory allowed confirmation and serotyping using

standardised and validated laboratory assays, with detailed characterisation of isolates using WGS, and sequence typing.

There are some limitations to our study. We were unable to serotype 15·8% of the 8316 invasive *H influenzae* isolates over the 14-year period, mostly because of failure to reculture at the local or national reference laboratory or because the local hospital did not submit the isolate to the national reference laboratory. Because Hia accounted for only 0·7% of serotyped isolates, it is probable that very few—if any—invasive Hia cases will have been missed. To account for isolates not typed, we compared incidence over time assuming non-serotyped cases had the same distribution as serotyped cases. This approach had minimal impact due to very small numbers in earlier years. In 2021–22, this correction would only increase confirmed invasive Hia cases from 19 to 21 estimated cases, with a respective increase in overall Hia disease incidence from 0·034 cases per 100 000 (unadjusted) to 0·038 cases per 100 000 (adjusted). Our surveillance only included invasive cases, but Hia also causes non-invasive disease, including non-bacteraemic pneumonia, which would not be captured in our surveillance. Additionally, further studies are needed to understand long-term outcomes of more severe presentations such as Hia meningitis in young children. Given the small number of Hia cases nationally, trends and outcomes should be interpreted with caution.

In England, there has been an increase in invasive Hia disease since 2016–17, with only a small reduction in cases among older adults during the 2020–21 COVID-19 pandemic restrictions, returning with the highest case numbers so far during 2021–22. This increase was mainly due to ST1511, ST23, ST56, and closely related single or double locus variants of these sequence types, with no evidence of capsular switching from Hib. Overall, Hia incidence remains low, with the highest incidence in infants, who typically presented with meningitis. Ongoing surveillance will be important to monitor recent trends, genomic patterns, and outcomes of invasive Hia disease in England, as well as elsewhere.

Contributors

SNL, MER, DJL, and NKF were responsible for the conceptualisation of the study. MB conducted the analysis. MB, EH, and ZA-C were involved in data curation and database management. MB, EH, and SNL had access to the epidemiological data. JCD and DJL conducted the genomic analysis and interpretation. MB and EH accessed and verified the data. MB wrote the first draft of the manuscript, which was edited and reviewed by all authors. All authors have seen and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

SNL conducts contract research on behalf of St George's University of London and the UKHSA for pharmaceutical companies, including vaccine manufacturers, but receives no personal remuneration. All other authors declare no competing interests.

Data sharing

Applications for relevant anonymised data should be submitted to the UKHSA office for data release at https://www.gov.uk/government/publications/accessing-ukhsa-protected-data.

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