The role of whole genome sequencing in revealing an HCV outbreak in Northern Ireland

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BACKGROUND

- The first hepatitis C virus (HCV) whole genome sequencing (WGS) assay to be used in the UK's National Health Service for clinical management was launched in 2019.
- The test determines the HCV genotype, subtype and NS3, NS5A and NS5B resistance pattern in a single test, which is agnostic to inputting subtype.
- WGS replaces the slower and more expensive sequential testing process of genotyping followed by resistance testing.
- The assay has been fully validated technically and clinically and is accredited by the UK's laboratory accreditation service (UKAS) [1, 2, 3]. Testing consists of the following steps: high throughput extraction, metagenomic RNA library prep, HCV-specific sequence capture, MiSeq sequencing, bioinfomatic pipeline giving technical and clinical reports.
- Technical reports represent a Quality Control step to detect possible contamination. They involve phylogenetic trees production of the current run plus the five previous runs for Core, E1, E2, NS3, NS4, NS5A, NS5B regions.
- Clinical reports are electronically issued to the referring clinician and may inform Direct Acting Antiviral drug regimen selection.

METHODS

- Northern Ireland routinely sends samples from all people newly-diagnosed with HCV for genotyping by WGS, giving high-density population coverage.
- Phylogenetic trees generated through routine bioinformatic analyses revealed a cluster of sequences from Northern Ireland patients, which was reported to Public Health. This report describes how phylogenetic analyses were used to identify and subsequently monitor the HCV outbreak in Northern Ireland

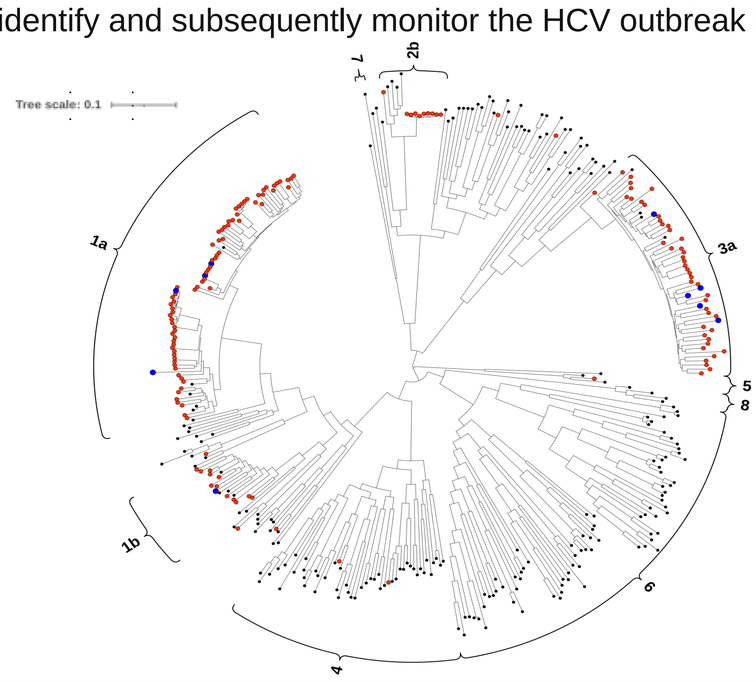


Figure 1. Example of a phylogenetic tree generated for Quality Control purposes from the current HCV whole genome sequencing run plus the five previous runs

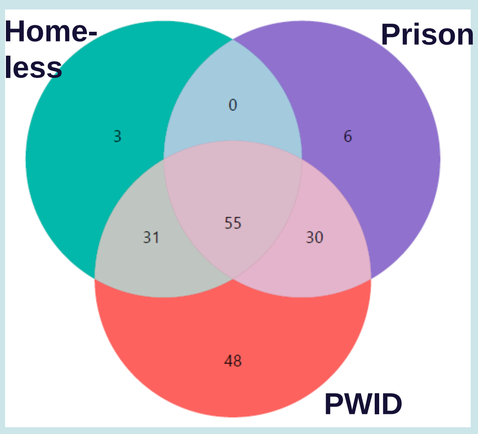
RESULTS

- In July 2020, small clusters of HCV sequences were identified through routine phylogenetic analyses. The requesting laboratory was noted to be Northern Ireland in all cases.
- Phylogenetic trees from the previous six months were therefore interrogated, revealing a cluster of 25 Northern Ireland sequences.
- At most recent analysis in August 2023, samples from 1068 Northern Ireland patients have been tested. Of these 466 (44%) are in a cluster. Table 1 shows the details of the nine clusters: 6 x genotype 1a, 1 x 2b and 2 x 3a.
- To characterise the clusters further, additional samples from the lab archive of Northern Ireland specimens received for HCV genotyping for surveillance purposes were included (February 2015 to March 2020). Several of these sequences added to existing clusters. The earliest sequences in clusters were from 2016.
- Eight dual HCV infections have been identified, demonstrating bridging between clusters: 2 x 1a/1a, 1 x 1a/1b, 3 x 1a/2b, 2 x 1a/3a.

Table 1. Details of the nine Northern Ireland (NI) clusters

Subtype	Cluster No.	Total	Non-NI	Year
1a	1	75		2017
	2	191	1	2016
	3	9		2017
	4	18		2017
	5	18		2016
	6	50	1	2020
2b	7	45		2017
3a	8	50		2017
	9	10		2018

Each column gives the number of unique patients. The final column gives the year of the earliest sample date for each cluster.



- Genotype 1a
- N=191 individuals
- Largest cluster
- 161 (86%) people who inject drugs (PWID)
- 91 (48%) contact with prison
- 89 (47%) homeless
- 55 (29%) PWID, homeless & prison contact
- 18 (9%) no known risk factor for HCV

Figure 2. Risk factors associated with HCV clustering (Cluster 2)

- Cluster membership was strongly associated with Belfast city centre postcodes, injecting drug use, homelessness / hostel residency, and a history of prison. Risk factors for the largest cluster, Cluster 2, are shown in Figure 2.
- Distinct patterns of HCV cluster growth were observed, with 1a and 2b clusters showing rapid growth. The 3a clusters were slow-growing (Figure 3)
- New trends in injecting drug use identified through the Unlinked Anonymous Monitoring survey of people who inject drugs (PWID) may partly explain the outbreak, with a switch from heroin to cocaine injecting, frequency of injecting and groin injecting, but no increase in use of needle and syringe exchanges.
- Future work will involve evaluation of reinfections, linkage to treatment outcomes, and generating time-stamped trees.

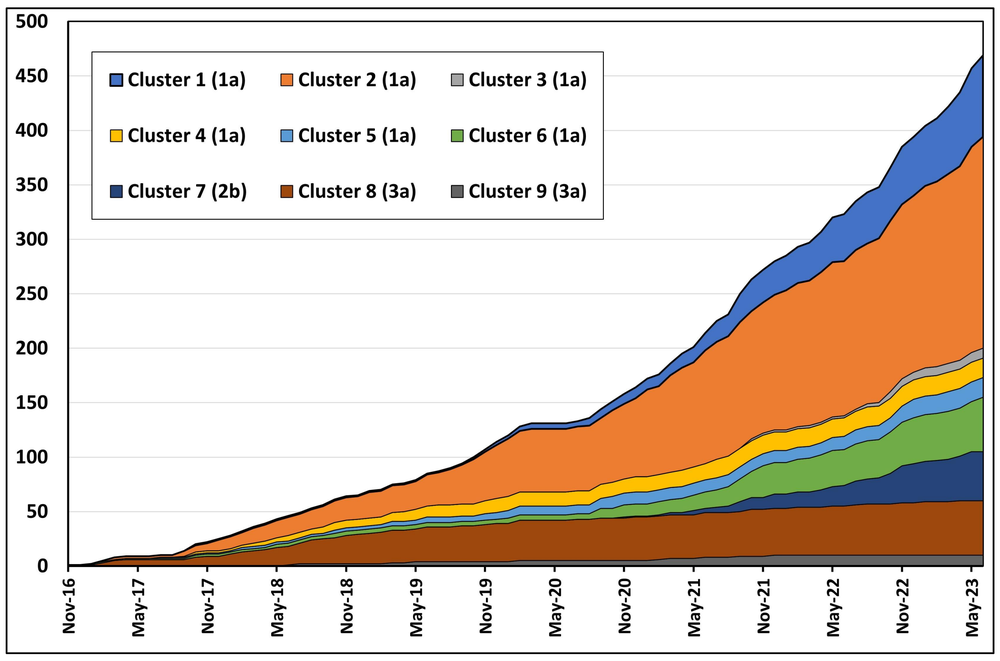


Figure 3. Growth curves for HCV clusters over time

CONCLUSIONS

- Use of sequencing in the clinical pathway revealed and facilitated monitoring of an HCV outbreak in real-time.
- High density population coverage of sequencing of Northern Ireland was likely important for outbreak identification. However, even representative sampling of a local cohort can deliver useful data to support public health programmes.
- Insights for targeting of HCV testing, awareness of demographic mixing patterns, and knowledge of incidence and re-infection rates can be improved through HCV phylogenetic analyses
- Phylogenetics will be important for monitoring progress towards and maintenance of HCV elimination within national programs.

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