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Phylogenetic Evidence for Intercity HCV Clusters of People Who Inject Drugs in India

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BACKGROUND

- Over 80% of the 71 million people globally infected with HCV live in low- and middle-income countries (LMICs).
- PWID bear a disproportionate HCV burden: 10 – 50 times that of the general population in some settings.
- Direct acting antivirals (DAAs) have revolutionized HCV treatment.
- To maximize cost-effectiveness of DAAs, strategies to treat vulnerable populations, such as PWID, are critical to minimize re-infection.
- Network-based treatment of PWID has been advocated as a strategy to reduce the risk of reinfection; however, understanding of PWID networks is incomplete and can vary from setting to setting.

OBJECTIVES

- Examine the phylogenetic clustering of hepatitis C sequences isolated from PWID across 4 different Indian cities at different epidemic stages to understand local transmission.
- Identify factors associated with clustering to inform prevention and treatment strategies.

METHODS

STUDY POPULATION

- Nested within a parent study which recruited PWID via respondent driven sampling (RDS) in 2016-17 as part of a cluster randomized trial (ClinicalTrials.gov identifier: NCT01686750).

Eligibility Criteria

1. 18 years of age or older
2. Provide verbal consent
3. History of injecting drugs for non-medicinal purposes in the prior 24 months
4. Present a valid RDS coupon

STUDY PROCEDURES

- All participants provided a blood sample and underwent an interviewer-administered electronic survey that captured information on demographics, network characteristics, risk behaviors, and access to HIV prevention and treatment services.
- HIV testing was performed on-site (three rapid tests) and results were delivered with pre- and post-test counseling.
- Biometric data was used to identify duplicates.
- All specimens were stored at the YRGCARE Infectious Disease Laboratory.

LABORATORY PROCEDURES

- HCV antibody testing was performed using the Genedia HCV ELISA 3.0 (Green Cross Medical Science, Korea) and confirmed by ARCHITECT Anti-HCV serology test (Abbott Diagnostics, USA).

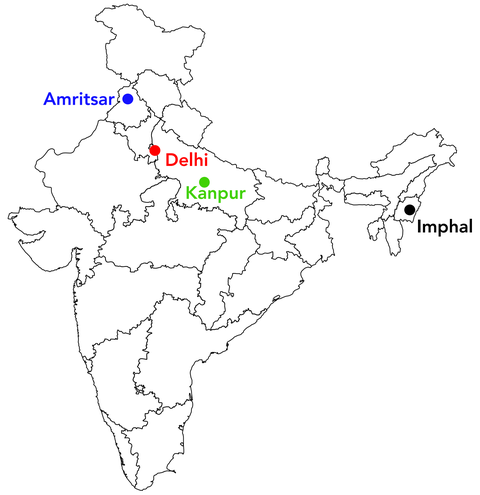

Figure 1. Map of India depicting the location of the 4 study cities: Amritsar (n=126), Delhi (n=128), Kanpur (n=138), and Imphal (n=94).

LABORATORY PROCEDURES *(METHODS CONTINUED)*

- HCV RNA quantification was performed using RealTime HCV Assay (Abbott Molecular, USA)
- HCV 5'UTR-core sequencing was performed on 486 HCV RNA positive samples from 4 cities (figure 1).
- All specimens with HCV RNA > 3000 IU/mL and availability of residual sample from these cities were sequenced.

STATISTICAL ANALYSES

- Phylogenetic inference was carried out using Maximum Likelihood methods in RaXML with 500 bootstrap replications (figure 2).
- Clusters were identified using ClusterPicker with posterior support and genetic distance thresholds of 70% and 4.5%, respectively.
- Machine learning models utilizing the Boruta wrapper of the random forest algorithm identified features predictive of clustering, further assessed by logistic regression.

ETHICAL CLEARANCE

- This study was approved by the Johns Hopkins Medicine and YR Gaitonde Centre for AIDS Research and Education institutional review boards.

RESULTS

- Mean p-distance for all sequences was 0.075
- 251 sequences fell into 19 transmission clusters
- Mean cluster size was 7.4 (range: 2-49)
- 8 dyads
- 6 large clusters comprised of > 10 samples
- 7 of 19 clusters contain samples from multiple cities

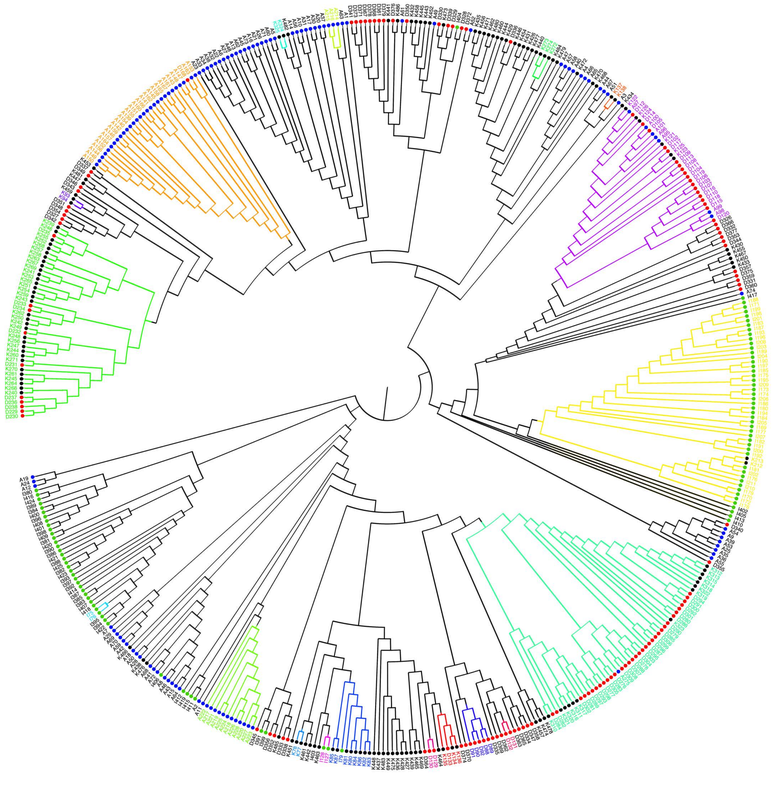

Figure 2. Cladogram depicting phylogenetic clustering of 486 HCV core sequences inferred by Maximum Likelihood. Evolutionary history was inferred in RaXML using 506 positions in the HCV core region under a GTR+G+I model and 500 bootstrap replications. Transmission clusters are denoted by branch and tip label color. Colored tip points denote city; Amritsar = blue, Delhi = red, Imphal = green, Kanpur = black.

	Amritsar (n=126)	New Delhi (n=128)	Kanpur (n=138)	Imphal (n=94)	Total (n=486)
Median Age (mean)	30.5 (31.1)	28 (29.2)	36 (37.3)	41 (39.9)	33 (34.0)
Proportion Male	100%	100%	100%	96.8%	99.4%
Shared Injection Equipment	77.0%	75.0%	56.5%	83.0%	71.8%
High School Education or Above	7.9%	0.8%	5.8%	51.1%	13.8%
Ever Tested for HIV	74.6%	46.9%	25.4%	83.0%	54.9%
Ever Tested for HCV	4.8%	1.7%	0%	71.2%	25.7%
# Clustered HCV Sequences	52 (41.3%)	74 (57.8%)	77 (55.8%)	48 (51.1%)	251 (51.7%)

Table 1. Baseline characteristics of study population (486 HCV-positive PWID from 4 Indian cities).

- Machine learning models trained on the data revealed that no history of HIV testing and living with friends were the most important predictors of whether or not a sequence clustered.
- State, residential zip code, injection zip code, time spent away from home, and antihistamine injection were the most important differentiating classifiers of the 19 transmission clusters in the machine learning model.
- Age, gender, and HIV status were not found to be predictive of clustering.

Predictor	Odds Ratio	95% CI	P-value
Never Tested for HIV	1.54	1.08 – 2.22	0.019
Live with Friends	4.02	1.32 – 12.24	0.014

Table 2. Factors associated with clustering identified by machine learning and logistic regression.

CONCLUSIONS

- More than half of samples clustered and had a relatively large median cluster size (compared to other reports from India), suggesting high-levels of interconnectedness between PWID in India.
- Nearly half of the clusters comprised of samples from multiple Indian cities, suggesting high levels of migration among PWID in India.
- To effectively eliminate HCV among PWID in India, networks may need to be defined by space (injection site) for network-based treatment efforts.
- Inter-city clustering suggests that HCV treatment programs in India should take migration into account, especially among vulnerable populations such as PWID, and plan nationally concerted efforts.

ACKNOWLEDGEMENTS

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