

# Poster # 451

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# Characterizing HIV Transmission Patterns among IDU Following an Outbreak in Sargodha, Pakistan

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### Abstract

**Background:** Over a one year period, HIV surveillance in Pakistan found a dramatic rise in HIV prevalence among injection drug users (IDU) in the city of Sargodha, evidence of an explosive outbreak. Sargodha has needle exchange programs in place; however, the common use of professional injectors and an innovative method of payment for drugs and services likely fueled the outbreak. We evaluated the molecular characteristics of HIV from IDU in Sargodha in order to determine whether the epidemic was caused by multiple introductions of HIV or from a single source.

**Methods:** Comparative phylogenetic analyses using the Neighbor-Joining method (Kimura 2-Parameter model) and bootstrap re-sampling as implemented in MEGA4, were performed on HIV *pol* protease and RT, and *env* gp41 sequences generated from dried blood spots collected from 400 IDU as part of the Canada-Pakistan HIV/AIDS Surveillance Project. Pairwise distances of clustered sequences were compared to those from Canadian transmission clusters and from Karachi IDU.

**Results:** Sequences from at least one genetic region were obtained from 151 (75.5%) of the 200 available HIV positive IDU DBS. Phylogenetic analysis revealed these to be subtype A1 (n=143) and CRF02\_AG (n=8). Further analysis revealed clustering of the sequences into two distinct infection groups: 142 within subtype A1 and 7 within CRF02\_AG. The mean distance between clustered Sargodha *pol* sequences was 0.4% and 1.45% for each cluster respectively, compared to 0.36% between clustered Ontario Polaris Seroneconverter Study sequences. Similarly, analysis of *env* gp41 revealed distances of 0.80% and 0.72% for Sargodha A1 and AG clusters, respectively, compared to 0.34% for sequences from another well characterized cluster. Our analysis also found that 7 of 17 sequences from Karachi grouped with the same distinct cluster.

**Conclusion:** Based on the findings of this analysis, 95% of Sargodha IDU specimens formed a single distinct cluster of highly related sequences, suggesting a single, recent, common source of infection, and a true outbreak among the Sargodha IDU population with links to Karachi IDU. Until the recent introduction of HIV, this had remained a high risk, low prevalence community. While interventions such as needle exchange programs may be reaching a proportion of Sargodha IDU, outreach programs must be implemented or expanded in order that those most susceptible, as well as peers and injectors, can better appreciate how to minimize risk.

### Background

- **Pakistan:**
  - Previously characterized as a low prevalence (<0.1%), high-risk epidemic
  - Recently upgraded to concentrated epidemic (within risk groups)
    - Larkana Outbreak: June 2003, 17/175 (9.7%) IDUs infected
    - Karachi: 23% HIV prevalence among IDUs
    - Punjab: Concentrated epidemics reported in 2005
  - Low level of knowledge about HIV/AIDS and prevention
    - High levels of STIs
    - Only 20% commercial sex workers always use condom
    - 20% Injection Drug Users (IDU) share needles & poor understanding of sharing)
    - Use of street injectors is common
- **Canada-Pakistan HIV/AIDS Surveillance Project (HASP):**
  - Second Generation Surveillance (biological & behavioral)
  - Goal of strengthening & expanding existing surveillance and monitoring (National & Provincial AIDS Control Programs, NGOs, Laboratories)
  - Recruitment sites in 12 cities in 4 provinces
  - Participants recruited (from each site):
    - 800 commercial sex workers:
      - 400 female
      - 200 male
    - 200 hijra / eunuch
    - 400 Injection Drug Users (IDU)

### Methods

#### Specimen Collection and Storage

- Dried blood spots were collected on Whatman 903 filter cards by finger prick. DBS were dried at ambient temperature for >3 hrs, then placed in paper envelopes. Shipping and further storage was at ambient temperature in Ziploc freezer bags containing desiccant.

#### Study Sample

- Nucleic acids were isolated from all available DBS from HIV positive Sargodha IDU (n=200), as well as HIV positive specimens collected in 2005 in Karachi as part of an earlier pilot study (n=34).
- Extractions were performed using the NucliSENS EasyMag system (BioMerieux) automated Bio-silica extraction. Two DBS were used when available (about 150µl whole blood).
- Nested RT-PCR was performed using custom primers targeting HIV *pol* protease, reverse transcriptase and *env* gp41

#### Molecular Analysis

- Sequences derived from DBS and from the HIV Sequence Database were phylogenetically compared using Neighbor-Joining analysis (Kimura 2-Parameter model) as implemented in the MEGA4.0. Sequences from Canadian and other infection clusters were included for comparison.
- Bayesian analysis of time-stamped taxa was performed using BEAST 1.5.2 under the GTR model (gamma + invariant sites) and chain length of 2 x 10<sup>9</sup>.

### HIV-1 Seroprevalence among Sargodha High-Risk Populations

Between 2005 and 2007, HIV prevalence among Sargodha IDU rose from 12% to 51.5%, the greatest increase of all the cities included in the study. Other risk groups (male, female and hijra sex workers) in Sargodha maintained low (<1%) prevalence in the same period.

Group	n=	Pos	% Pos	Indeterminate
IDU	400	206	51.5	2
FSW	400	1	0.3	
HSW	200	1	0.5	
MSW	200	2	1.0	
<b>Overall</b>	<b>1200</b>	<b>210</b>	<b>17.5</b>	

### PCR/Sequencing (n=200)

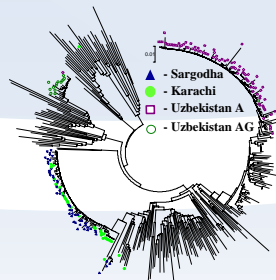
Genetic Region	PCR Positive
<i>pol</i> (1200 nt)	45
RT (650 nt)	113
<i>env</i> gp41 (450 nt)	126
<b>Total Positive (one or more regions)</b>	<b>151 (75.5%)</b>

### Sargodha IDU HIV Subtype

Subtype	n
subtype A1	143 94.7%
subtype AG	7 4.6%
Other A-Recombinant	1 0.7%

We were able to obtain sequence information from 75.5% of Dried Blood Spots from HIV positive IDU. The vast majority were subtype A1. Karachi specimens from a 2005 pilot study yielded sequence information from 34 of the 35 available DBS, again with the majority being subtype A1.

### Phylogenetic Analysis of Sargodha, Karachi and Uzbekistan HIV-1 Protease



Sargodha HIV protease sequences (n=56) were evaluated in the context of sequences from Karachi (n=32) as well as sequences from an HIV outbreak among Uzbekistan IDU (n=140), outbreak of a West African nonfounder of HIV-1 in Tashkent, Uzbekistan. *Curr Jk et al. J AIDS* 2005; 19(5):576-3

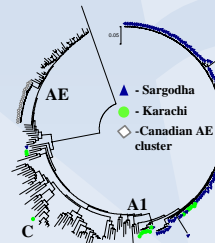
#### Mean pairwise distance between clustered sequences:

Cluster	Distance
Sargodha A1 (main cluster)	0.42 %
Karachi A1 (main cluster)	0.31 %
Uzbekistan A1 (main cluster)	0.50 %
Sargodha outliers (AG)	0.70 %
Uzbekistan AG	0.66 %

The mean distance between Sargodha and Karachi sequences in the main A1 cluster was 0.43%—the mean distance between the other groups ranged from 4% (Sargodha-AG vs. Sargodha and Karachi A1) and higher. The mean distance between unrelated subtype A & A1 sequences was 5.96%. The majority of Sargodha (92.8%) and Karachi (87.5%) sequences were in the main A1 cluster.

### Results

#### Phylogenetic Analysis of HIV-1 *env* gp41 Sequences from Sargodha and Karachi IDU

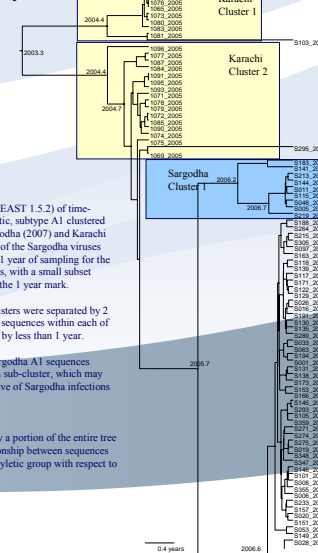


The *env* gp41 region was analyzed in the context of a Canadian AE transmission cluster.

Cluster	Distance
Sargodha A1 (main)	0.70 %
Karachi A1 (main)	0.50 %
Karachi A recombinant	0.85 %
AE Transmission Cluster	0.20 %

The mean distance between Sargodha and Karachi sequences in the main A1 cluster was 0.71%. The mean distance between other groups ranged from 3% (Sargodha & Karachi A-recombinants vs. the A1 cluster) and higher. The majority of Sargodha (99.2%) and Karachi (89.3%) sequences were in the main A1 cluster.

#### BEAST Analysis



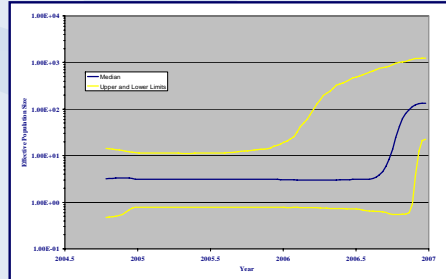
Bayesian analysis (BEAST 1.5.2) of time-stamped, monophyletic, subtype A1 clustered sequences from Sargodha (2007) and Karachi (2005). The MRCA of the Sargodha viruses was within less than 1 year of sampling for the majority of sequences, with a small subset originating just over the 1 year mark.

Two Karachi sub-clusters were separated by 2 years. However, the sequences within each of these were separated by less than 1 year.

A small subset of Sargodha A1 sequences (7.3%) also formed a sub-cluster, which may represent the first wave of Sargodha infections detected in 2005.

(Note that this is only a portion of the entire tree examining the relationship between sequences from the A1 monophyletic group with respect to sampling time.)

#### Bayesian Skyline Analysis of Effective Population



Bayesian Skyline analysis (BEAST 1.5.2) was used to estimate the effective population size of the monophyletic HIV subtype A1 cluster (Sargodha and Karachi) based on HIV *env* gp41 sequences. A rapid increase in the effective population was observed between 2006 and 2007, supporting epidemiological findings. Similar analysis of only the Karachi data lacked power to detect the earlier outbreak in that city.

### Conclusions

The monophyletic subtype A1 clustering of the majority of Sargodha IDU sequences indicates these infections originated from a common source (founder), followed by rapid epidemic spread among this population, supporting epidemiological findings. The small A1 sub-cluster (7.3%) of Sargodha IDU specimens may have originated from an earlier introduction of the same founder virus.

The majority of Karachi HIV specimens collected in 2005 are part of the main Sargodha cluster, indicating that the two epidemics share the same recent common origin (MRCA).

In recent years, Pakistan has experienced several "localized" HIV outbreaks among IDU. The evidence presented here demonstrates that the epidemics in Sargodha and Karachi, cities 1000 km apart, share a common origin. Consequently, disparate epidemics in other regions may form part of the same overlapping outbreaks found in Sargodha and Karachi.

Understanding the dynamics of HIV transmission in Pakistan, combined with existing knowledge of similar outbreaks elsewhere in the world, can lead to more effective prevention strategies.

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