Applying Genotype Cluster Analysis Techniques When Investigating Specific Genomic Signatures

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ABSTRACT

Background: The New Jersey Department of Health continues its partnership with The Public Health Research Institute (PHRI) to provide *IS6110* Restriction Fragment Length Polymorphism (RFLP) on all culture positive *M.tb* samples, as part of its universal genotyping project. In 2017, a pediatric *M.tb* culture was identified as a possible DNA match to a 2014 genotype cluster. All samples were submitted to the national genotyping laboratory for standard genotyping sequencing. The 2017 pediatric *M.tb* culture submitted for *IS6110* RFLP, Spoligotpyping and MIRU-24, returned a genomic signature matching the 2014 genotype cluster, identified as GENType G12029.

Methods: For 2017 and 2014, LJ slant cultures were submitted to PHRI, for strain genotyping using standard *IS6110* methods. The RFLP patterns were compared against the archived PHRI image database (over 10,000 images, 31,000 *M.tb* isolates) for strain identification. The extracted DNA was then sent to the national genotyping laboratory for sequencing and comparison against the archived national *M.tb* genotyping database.

Results: The five case-patient 2014 cluster and one case-patient 2017 sample generated matching *IS6110* RFLP patterns labeled AI310 (10 bands). All samples submitted to the national genotyping laboratory returned matching genomic signatures, resulting in GENType G12029 designation. National distribution patterns identify GENType G12029 as a seven case-patient cluster with 86 percent (n= 6) of the case-patients epidemiologically linked within the same NJ household.

Conclusions: The 2017 GENType G12029 cluster investigation identified a direct epidemiological link, via a previously shared home address, between the 2014 and 2017 case-patients. The follow-up cluster investigation for the 2014 genotype cluster revealed that two of the case-patients acknowledged close contact with a separate family that had shared a common living area, but were subsequently lost to follow-up. All adult case-patients identified in NJ listed Mexico as their country of origin. In total, investigation for GENType G12029 identified six culture confirmed case-patients, one clinically confirmed pediatric case, and no evidence of ongoing transmission occurring within the community.

BACKGROUND

- Standard based *IS6110* Restriction Fragment Length Polymorphism (RFLP) is available for all culture positive *M.tb* specimens collected in NJ, prior to their arrival at the National Genotyping Laboratory in Michigan.
- The five case-patient 2014 cluster and one case-patient 2017 sample generated matching *IS6110* RFLP patterns labeled AI310 (10 bands).
- 2017 contact investigation was used to expanded on the 2014 G12029 cluster investigation.
- We describe our recent experience in which the timely availability of *IS6110* RFLP Analysis was used in verifying epidemiological links between six cases in the AI310 RFLP cluster.

METHODS

- Lowenstein - Jensen slant cultures were immediately retrieved for strain genotyping using standard based *IS6110* RFLP methods.
- Resulting images were compared against the archived PHRI image database (> 10,000 images; 31,000 *M.tb* isolates) for strain identification.
- The analyzed DNA was then sent to the national genotyping laboratory for sequencing and comparison against the archived national *M.tb* genotyping database.
- National genotyping data provided Spoligotyping lineage, 24-Locus MIRU-VNTR, PCR and GENTypes for comparison within the national surveillance database.

RESULTS

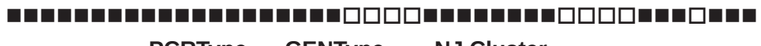
- PHRI analyzed the specimen DNA in all 6 cases and confirmed the *IS6110* RFLP as RFLP cluster AI310 (10 bands) ***(fig.2)***
- Results from the National Genotyping Lab returned matching PCR Type18485 designation for the one pediatric case and the five adult household cases.
- National distribution patterns identify GENType G12029 as a seven case-patient cluster with 86 percent (n= 6) of the case-patients epidemiologically linked within the same NJ household***. (fig.1)***
- The six case-patient cluster investigation found Mexico as a matching country of origin for all adult cases.

CONCLUSIONS

- The 2017 GENType G12029 cluster investigation identified a direct epidemiological link, via a previously shared home address, between the 2014 and 2017 case-patients***. (fig.3)***
- Follow up investigation on the 2014 pediatric cluster found that two of the case-patients acknowledged close contact with a separate family that had shared a common living area, but were subsequently lost to follow-up.
- Investigation for GENType G12029 identified six culture confirmed case-patients, one clinically confirmed pediatric case. ***(fig.3)***
- No evidence of ongoing transmission occurring within the community.
- National Genotyping surveillance data aided in the confirmation of epidemiological links for all culture confirmed cases in this cluster. ***(fig.1,3)***

Fig. 1: GENType G12029 Surveillance Summary

Spoligotype	24-Locus MIRU-VNTR
777777607760731	124326143226 243224123228



PCRType	GENType	NJ Cluster
18485	12029	NJ_0284_001

Fig. 3: GENType G12029 Cluster Association Matrix

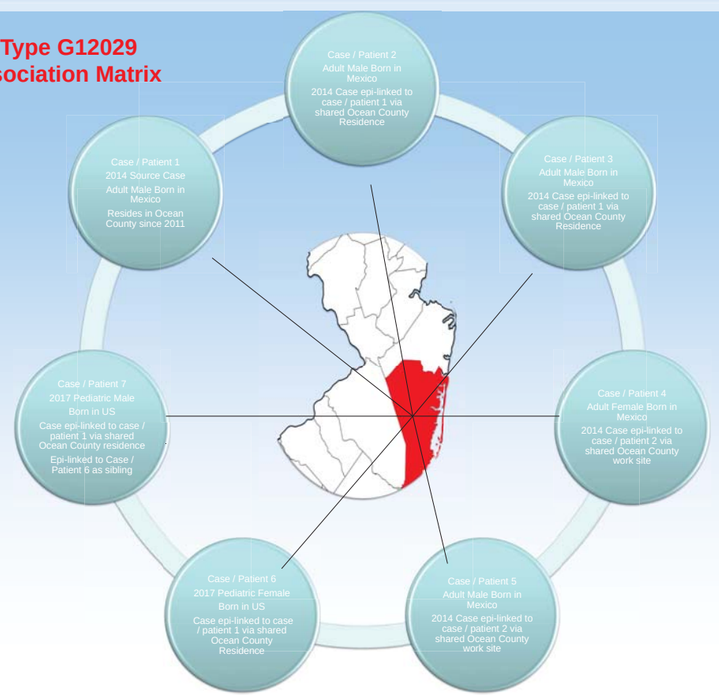
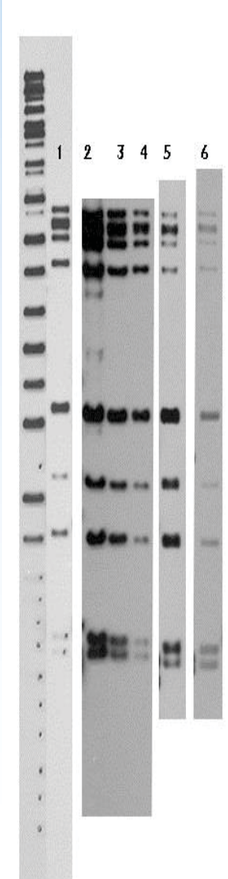


Fig. 2: *IS6110* RFLP AI310 (10 bands)



AI310 cluster

1. 2014 Case / Patient 1
2. 2014 Case / Patient 2
3. 2014 Case / Patient 3
4. 2014 Case / Patient 4
5. 2014 Case / Patient 5
6. 2017 Pediatric Case / Patient 6

CASE STUDY

In 2014, *IS6110* RFLP AI310 (10 bands) was identified by The Public Health Research Institute (PHRI) within 5 samples that had been submitted for genotyping. The initial 2014 RFLP AI310 cluster investigation found that all five case-patients returned matching GENType designations labeled G12029. All five case-patients had also resided within the same residence during the infectious period of the adult household source case.

In 2017, The Public Health Research Institute (PHRI) identified a sixth DNA match to the *IS6110* RFLP AI310, G12029 designation type. The RFLP AI310 cluster investigation was reinstated to include this new case. The 2017 G12029 cluster investigation found that this new case was a pediatric US born child with parents born in Mexico, matching the previous five case-patients . This new case belonged to a family first identified within the 2014 cluster investigation, then subsequently lost to follow-up. The 2017 cluster investigation identified six culture confirmed case-patients, and one clinically confirmed pediatric case. To date, no additional epidemiological links have been identified, and no evidence has been found of any ongoing transmission occurring within the community.