

Ten Year Genotype Cluster Overview using IS6110-Based Restriction Fragment Length Polymorphism Analysis Techniques



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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. Beginning in 2008, a total of eight *M.tb* cultures have been identified as matching on genetic cluster with seven of the eight case-patients (88%) reported within the same geographic area. All samples were submitted to the national genotyping laboratory for standard genotyping sequencing. Each of the eight *M.tb* cultures submitted for *IS6110* RFLP, Spoligotyping and MIRU-24, returned the exact genomic signature identified as GENType G14581.

Methods: Lowenstein - Jensen 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, 41,000 *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 eight *M.tb* cultures submitted generated matching *IS6110* RFLP patterns labeled BW516 (8 bands). All samples submitted to the national genotyping laboratory returned matching genomic signatures, resulting in GENType G14581 designation. National distribution patterns identify GENType G14581 as an eight case-patient cluster with 63 percent (n= 5) of the case-patients having been reported as “homeless within the past year”, at time of diagnosis.

Conclusions: From 2008–2018, the GENType G14581 cluster investigation identified direct epidemiological links, via the food pantry amenities of local rescue mission in one NJ township. The follow-up cluster investigation revealed that of the eight-total case-patients identified in NJ, seven (88%) were reported out of the same NJ township, with five of those eight case-patients (63%) acknowledging previous contact with each other. Over a 10 year span, investigation into GENType G14581 has identified eight culture confirmed case-patients sharing common living routines centered on a local rescue mission and its amenities. Sixty three percent (n= 5) of the case-patients identified were reported as “homeless within the past year” at time of diagnosis, with no direct evidence of ongoing transmission occurring within the facility.

BACKGROUND

- Standard based *IS6110* RFLP is available for all culture positive *M.tb* specimens collected in NJ, prior to their arrival at the National Genotyping Laboratory in Michigan.
- From 2008–2018, eight active *M.tb* cases, reported in NJ, were identified as *IS6110* RFLP strain BW516 (15 bands). **fig.1.**
- Seven of the eight case-patients (88%) reported out of the same NJ Township.
- Subsequent genotype cluster investigations produced definitive epi-links among five of the seven case-patients (71%) identified within that NJ township.
- Whole – genome single nucleotide polymorphism (wgSNP) analysis was performed in 2018, to further assess the genetic relatedness of the isolates.
- Described is recent experience in which the timely availability of *IS6110* RFLP Analysis was used in verifying epidemiological links between all case-patients identified.

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; 41,000 *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.
- Whole - genome single nucleotide polymorphism (wgSNP) analysis was performed on six of the eight MTB cultures (75%) submitted between 2008 through 2018. **fig.2.**
- No cultures were available for two cultures reported in 2009 and 2010, respectively. **fig.2.**

RESULTS

- All eight positive *M.tb* cultures generated a matching *IS6110* RFLP pattern labeled BW516 (15 bands), with samples returning full genotyping sequence and subsequent whole genome sequencing. **fig.1.**
- Results from the National Genotyping Lab returned PCR Type PCR00201, with GENType G14581. **fig.1.**
- Those samples submitted prior to 2009 had not received MIRU2 or GENType designation. **fig.1.**
- National distribution patterns identify GENType G14581 as an eight case-patient cluster with 63 percent (n= 5) of the case-patients having been reported as “homeless within the past year”, at time of diagnosis.
- GENType G14581 was analyzed using whole-genome single nucleotide polymorphism (wgSNP) analysis techniques. **fig.2.**
- wgSNP analysis expands coverage of the genome to ~ 90%, compared to ~1% genetic coverage with conventional genotyping methods.

CONCLUSIONS

- From 2008–2018, investigation into GENType G14581 identified eight culture confirmed case-patients sharing common living routines centered on a local rescue mission.
- Five of those eight case-patients (63%) acknowledged previous contact with each other.
- These same five case-patients (63 %) were also reported as “homeless within the past year”, at time of diagnosis, with no direct evidence of ongoing transmission occurring within the facility.
- wgSNP analysis illustrated the genetic relatedness between G14581 case-patients, 16RF3155 and 17RF6476. **fig.2**
- wgSNP analysis identified the genomic signature of both case-patients as the “most recent common ancestor”, serving as a reference point for examining the direction of change within the genome. **fig.2.**
- wgSNP analysis identified a 4 SNP sub-clustering between select NJ and PA case-patients. **fig.2.**

CASE STUDY

Beginning in November 2008, GENType G14581 was first identified in case-patient 09RF0330. This case-patient had a documented history of homelessness, as well as drug and alcohol use at time of diagnosis. *IS6110* RFLP BW516 was identified in DNA analysis. **fig.3, fig.4.**

In September of 2009, case-patient 10RF4086 was identified as matching in *IS6110* RFLP and PCRType sequence to the November 2008 case-patient. This new case-patient had also been found to have a documented history of homelessness and alcohol use at time of diagnosis. The initial contact investigation identified case-patient 10RF4086 as the biological brother to 09RF0330. **fig.3, fig.4.**

In November 2009, case-patient 09RF4775 was identified as matching on *IS6110* RFLP and PCRType sequence to the BW516 cluster. This case-patient was reported with a history of homelessness and alcohol use at time of diagnosis, however no direct epi-links were found to match case-patients 09RF0330 and 10RF4086. **fig.3, fig.4.**

Moving forward to February 2016, case-patient 16RF3155 was identified as GENType G14581 and matching the BW516 cluster. This 2016 case-patient reported a documented history of homelessness and substance abuse at time of diagnosis, similar to the 2009 and 2010 case-patients. 2016 cluster investigation yields epi-links to case-patients 09RF0330, 10RF4086, and 09RF4775 directly. These three case-patients were verified as acquaintances from the food pantry housed within a local rescue mission. **fig.3, fig.4.**

In March 2016, 16RF3152 was identified as matching on *IS6110* RFLP and GENType to the BW516 cluster. This case-patient reported no history of drug and alcohol use at time of diagnosis, however was positively identified as a recipient of services from the same local rescue mission as the previous case-patients within GENType G14581. Cluster investigation yielded no direct epi-links to the four previous case-patients within the BW516 cluster. **fig.3, fig.4.**

January 2017, 17RF2957 was identified as matching on *IS6110* RFLP and GENType to the BW516 cluster. This case-patient reported a history of homelessness, but no known drug and alcohol use at time of diagnosis. The 2017 cluster investigation yielded epi-links to 16RF3152 and 16RF3155. **fig.3, fig.4.**

May 2017, 17RF6476 returned a matching *IS6110* RFLP and GENType as the previous case-patients within the BW516 cluster. This case-patient died unrelated to TB disease and the cluster investigation yielded no known epi-links to the six previous case-patients listed within the cluster. **fig.4.**

July 2018, 18RF6496 was reported with a documented history of homelessness and drug and alcohol use at time of diagnosis. *IS6110* RFLP and GENType matched the BW516 cluster, and to date, there are no direct epi-links found to any of the previous case-patients. **fig.4**

January 2019, wgSNP analysis on G14581 was made available to NJ. The wgSNP distinguished two NJ case-patients sub-clustering 4 SNPs away from two reported PA case-patients. **fig.2.**

Following the inclusion of whole genome sequencing (WGS) an inter-jurisdictional cluster investigation has been initiated between NJ and PA. At the current time no additional epi-links have been identified. Any new epi-links verified from this cluster investigation will be a direct result of the combined efforts of contact investigation techniques, the timely availability of *IS6110* RFLP, and WGS data to recognize previously unknown genetic relationships among specific case-patients.

Fig.1.

GENType G14581 Patient History

Case	Spoligotype	MIRU	MIRU 2	Cluster ID	PCR Type	GENType	IS6110 RFLP
09RF0330	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	N/A	BW516
10RF4086	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	N/A	BW516
09RF4775	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	N/A	BW516
16RF3155	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	G14581	BW516
16RF3152	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	G14581	BW516
17RF2957	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	G14581	BW516
17RF6476	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	G14581	BW516
18RF6496	77777777720771	224325153323	233434423336	NJ_0075_001	PCR00201	G14581	BW516

Fig.2.

wgSNP Association Matrix

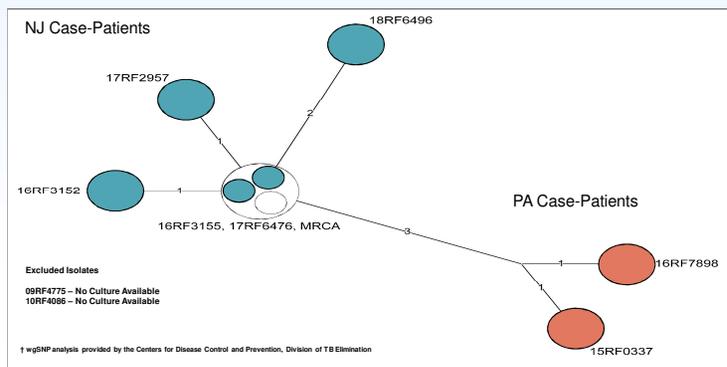


Fig.3.

IS6110 RFLP BW516 Association Matrix

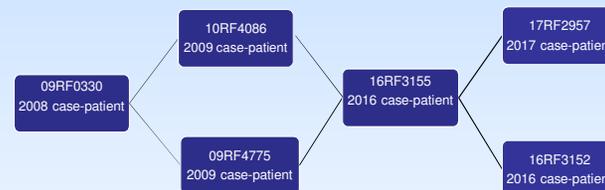
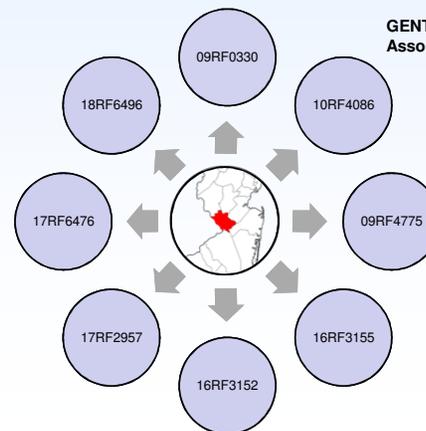


Fig.4.

GENType G14581 Association Matrix



¹ wgSNP analysis provided by the Centers for Disease Control and Prevention, Division of TB Elimination