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Outbreak of pyrazinamide-monoresistant tuberculosis identified using genotype cluster and social media analysis

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SUMMARY

SETTING—Monoresistance to pyrazinamide (PZA) has infrequently been associated with *Mycobacterium tuberculosis*.

OBJECTIVE—To report an outbreak of PZA-monoresistant *M. tuberculosis* in Virginia involving two genotype clusters from December 2004 to August 2010.

RESULTS—Thirty cases were identified involving a predominantly young, US-born population with histories of substance use and incarceration and a large proportion of children aged <15 years ($n = 6$, 20%); of these, 23 cases (77%) were culture-confirmed as *M. tuberculosis* complex. DNA fingerprinting and molecular analysis of the PZA resistance gene, *pncA*, demonstrated a clonal strain that was not *M. bovis*. Genotypic data provided the initial link between seemingly unrelated cases, and helped reveal a historic genotype cluster of cases from 2004. Further genotype cluster and contact investigation procedures, including the novel use of the social networking website Facebook.com, revealed additional links between the 2004 and 2009 genotype clusters and described an ongoing, extensive outbreak necessitating an enhanced screening and treatment protocol for contacts.

CONCLUSIONS—This outbreak demonstrates how tuberculosis can spread through a young, vulnerable population. The use of genotypic data and the novel incorporation of social media investigations were critical to understanding the settings and context of infectivity.

Keywords

drug resistance; *pncA* protein; Virginia; pediatrics; social media

IN 2009, AN OUTBREAK of *Mycobacterium tuberculosis* heralded by monoresistance to pyrazinamide (PZA) was identified in a metropolitan area of Virginia among a young US-born population. PZA is a first-line anti-tuberculosis medication with unique bactericidal properties against intracellular semi-dormant bacilli, and an essential component of the

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quadruple-drug regimen for short-course anti-tuberculosis treatment.¹ Monoresistance to PZA (PZA_{MR}) has historically been associated with *M. bovis*, a *M. tuberculosis* complex organism with inherent resistance. However, over the past 15 years PZA_{MR} *M. tuberculosis* has been increasingly reported.^{2–6}

PZA susceptibility testing by conventional phenotypic methods remains technically demanding due to the need to replicate in vitro the acidic environment required for PZA activity. The radiometric method is considered a reference method; however, most laboratories use the non-radiometric BACTEC™ MGIT™ 960 (BD, Sparks, MD, USA) system. The reproducibility and validity of MGIT960 results have been variable,⁷ with some groups reporting high concordance with the radiometric method,⁸ and others notable discordance.⁹ Molecular assays have been developed for the *pncA* gene, a 561 base-pair gene encoding for the enzyme pyrazinamidase. Mutations within this gene or its promoter region may render the mycobacteria unable to convert PZA into the active drug, pyrazinoic acid. Approximately 85% correlation between *pncA* mutations and phenotypic results has been reported.¹⁰

When considering the spread of *M. tuberculosis* within a community, universal genotyping can be a valuable tool for tuberculosis (TB) control programs. The Centers for Disease Control and Prevention (CDC) supported National Tuberculosis Genotyping Service (NTGS) provides spoligotyping, mycobacterial interspersed repetitive units-variable numbers of tandem repeat (MIRU-VNTR) typing, and, on request, insertion sequence (IS) 6110 restriction length fragment polymorphism (RFLP) on TB isolates in the United States. Genotyping combined with epidemiologic investigations may aid conventional contact investigations by revealing unsuspected relationships and transmission settings.¹¹ Social media websites such as Facebook.com have facilitated dissemination of health information; however, their use during genotype cluster investigations for TB has yet to be reported.¹²

In the present study, we report the investigative procedures involved in this outbreak of PZA_{MR} TB in Virginia and describe how genotype cluster investigation impacted conventional contact investigation procedures. An unexpectedly extensive outbreak was revealed, necessitating an enhanced screening and treatment protocol that uncovered additional latent and active cases.

STUDY POPULATION AND METHODS

Definitions

TB cases included those with *M. tuberculosis* culture-confirmed disease. Clinical cases included contacts of TB cases with positive tuberculin skin test (TST) results and suggestive symptoms and/or chest radiographic findings but negative cultures. Cases were further categorized based on the site of disease, including pulmonary/lymphatic intra-thoracic disease, extra-pulmonary disease or both. The index case refers to the initial case detected. The source case refers to the patient thought to have infected the index cases. An epidemiologically linked case refers to someone connected to a TB case in the contact investigation via shared physical locations (workplaces, jail, home), or persons named by health department personnel or as documented in medical records. Matching RFLP patterns

were those with equal numbers of bands on gel electrophoresis and matching molecular weights.

Study design and population

The outbreak investigation was conducted in 2009–2010 by the Virginia Department of Health (VDH), and included concurrent contact investigations and retrospective genotype cluster investigations in a metropolitan area of Virginia. TB cases were included if their isolates demonstrated PZA_{MR} with matching genotypes between December 2004 and August 2010. Clinically diagnosed cases were also included if they were epidemiologically linked to an outbreak-associated culture-confirmed case.

Investigative procedures

All newly diagnosed TB cases were assessed by local health departments utilizing standard contact investigation procedures.¹³ A genotype cluster investigation was initiated when two seemingly unrelated cases were found to match each other and an existing genotype cluster within the same metropolitan area. In addition, the cases had an uncommon drug susceptibility testing (DST) profile for PZA_{MR}. The genotype cluster investigation included retrospective record review, investigations at local jails, re-interview of available cases, and interviews with public health nurses and outreach workers. The VDH database was queried retrospectively to look for additional TB cases with PZA_{MR}. When a historic genotype cluster from the same geographic area was identified with matching DST profiles, further investigations were pursued, including utilization of the social media website, Facebook.com, to identify connections between the 2004 and 2009 clusters. TB cases were searched by name on the website, and their respective lists of publicly visible ‘friends’ were examined to identify mutual acquaintances. When TB cases were found to have a mutual ‘friend’, they were assumed to share a plausible epidemiologic link.

Laboratory methods

All TB isolates underwent first-line DST at the state laboratory via the automated Bactec MGIT960 system. Resistance to PZA was initially identified using the MGIT PZA kit (critical concentration: 100 µg/ml) (Bactec MGIT 960 PZA kit package insert, BD, 2009). For this study, confirmation of PZA resistance was pursued by minimum inhibitory concentration (MIC) testing using two-fold series of PZA concentrations from 25 µg/ml to 800 µg/ml.¹⁴ When any PZA resistance was detected, speciation to distinguish between *M. tuberculosis* and *M. bovis* was performed using nitrate reductase and niacin accumulation assays.¹⁵

Further confirmatory testing via genetic sequencing of the *pncA* gene among a subset of isolates found to be PZA-resistant was performed as previously reported.¹⁴ DNA fingerprinting techniques included spoligotyping and MIRU-VNTR sequencing as per standard protocol, and a subset of isolates also underwent MIRU-2 analysis and IS6110 RFLP testing at the Michigan State Laboratory at the request of the VDH.¹¹

Statistical analysis

Clinical and epidemiologic variables were assessed using simple frequencies, stratified by genotype cluster and method of TB diagnosis (clinical or culture-confirmed). All analyses were performed using SPSS Statistics v20.0 software (IBM, Armonk, NY, USA).

Ethics

All information was collected as part of a public health investigation in accordance with the data security and confidentiality guidelines set forth by the VDH.¹⁶ This study was exempt from human subjects research review.

RESULTS

Index cases

In September 2009, two index cases with culture-confirmed TB and PZA_{MR} were identified, one adult and one young child. Routine contact investigation did not initially identify epidemiologic links between these two cases. However, contact investigation for the index adult revealed three additional cases, including a second pediatric case and an adult presumed to be the source case linking the two index cases (Figure 1).

Genotype cluster investigation

In December 2009, the genotype results for the two index cases were found to match each other as well as another contemporary cluster (2009 cluster, $n = 14$). Database review of all PZA_{MR} TB cases from the previous decade identified another historic genotype cluster ($n = 9$) from 2004 located in the same geographic area. By spoligotyping, the 2004 cluster (67777760776071) differed from the 2009 cluster by one spacer (67773760776071); however, 24-locus MIRU-VNTR analysis revealed complete matches between the two clusters, and IS6110 RFLP analysis revealed matching 12-band fingerprints for all cases except one. Based on the NTGS nomenclature system, each cluster was assigned unique names due to differing spoligotypes (Table 1).

To determine if the PZA_{MR} TB cases represented one continuous outbreak, additional investigation procedures were conducted. Self-reported behaviors, including substance use and frequent attendance at large social gatherings, prompted further investigation on Facebook.com, which importantly revealed the only plausible epidemiologic links between members of the 2004 and 2009 clusters, and further confirmed links within the 2009 cluster. These findings suggested that, despite routine procedures, there was inadequate containment of the outbreak, beginning with the 2004 genotype cluster.

Laboratory confirmation of PZA resistance

Conventional MGIT960 DST revealed that all isolates from culture-confirmed cases among the two clusters demonstrated PZA_{MR}. Nitrate reductase and niacin accumulation assays confirmed speciation as *M. tuberculosis*. Quantitative resistance testing revealed a PZA MIC of >800 µg/ml, indicating high-level resistance. *pncA* gene sequencing detected an identical mutation among all isolates tested: the deletion of nucleotide G at position 290, conferring a frame shift mutation with subsequent amino acid changes. The hallmark genetic

polymorphism associated with *M. bovis*, a point mutation from C to G at position 169, was not detected.

Enhanced evaluation and treatment protocol

All identified contacts of TB cases underwent chest radiograph, regardless of baseline TST result. Second, although latent tuberculous infection (LTBI) was diagnosed using standard practice, due to concerns about rapid progression from exposure to disease the enhanced protocol recommended that all contacts initiate LTBI treatment after excluding active disease while awaiting repeat TST results ('window period LTBI treatment'), akin to the standard practice for children aged <5 years. Directly observed preventive therapy (DOPT) was provided for all children and adults. Those who refused or were unable to complete LTBI treatment were recommended to undergo enhanced monitoring via periodic follow-up for 2 years after exposure.

Among the 168 contacts evaluated, 11 (7%) had active disease, including 5 (46%) children with culture-negative TB, 2 (18%) adults with culture-negative TB and 4 (36%) adults with smear/culture-positive disease; 19 (11%) were found to have LTBI. Of these, 14 (74%) were initiated on LTBI treatment and 6 (32%) completed the course.

Characteristics of outbreak cases

Thirty cases were identified among the two genotype clusters (Figure 2), representing 10.3% of all regional TB cases.¹⁷ The majority of the cases were adults with culture-confirmed pulmonary TB ($n = 16$, 53%), of whom nine (56%) had cavitary disease. Seven (23%) cases, including five (17%) children, all from the 2009 cluster, were culture-negative and diagnosed on clinical grounds (Table 2). All children had isolated pulmonary/intra-thoracic disease and were exposed in the home. The proportion of annual pediatric TB cases from this region attributed to this outbreak from 2009 to 2010 was 55% (Figure 3).¹⁷

Nearly all of the cases ($n = 28$, 93%) were US-born. One patient (3%) was human immunodeficiency virus infected, three patients (10%) had diabetes mellitus, two of whom also had end-stage renal disease, and three others (10%) were known to be infected with the hepatitis C virus. Substance use in the preceding year was self-reported by 12 patients (40%), and included excessive alcohol, marijuana, or cocaine use, and/or other injection drug use.

DISCUSSION

We describe two linked TB genotype clusters heralded by their DST profiles, which revealed an uncommon finding of PZA_{MR}. There were four main findings from this work. First, we wish to emphasize that PZA_{MR} *M. tuberculosis* was the cause of this extensive outbreak; confirmatory *pncA* sequencing excluded involvement of *M. bovis*. In support of a clonal strain, all tested isolates had an identical frame-shift mutation with concomitant high-level PZA resistance; this is unusual, given the typical heterogeneity of *pncA* mutations conferring PZA resistance.^{2-4,10,18,19}

Second, genotype cluster investigations and the use of social media established plausible epidemiologic links between cases from the 2004 and 2009 clusters that had not been identified during routine contact investigations, suggesting that a social network linked the two genotype clusters, fueling a continuous outbreak. Traditional contact investigation techniques focused on the home, workplace and common social environments helped identify potential contacts. Limitations of this approach have been reported, particularly when applied to populations at high risk for TB transmission, including adults with substance dependence, who may be unwilling or unable to report contacts.²⁰ Social media have been employed to publicize health information, and have increasingly been used as an ‘infodemiology’ tool to monitor online behavior to evaluate trends in illnesses such as influenza.²¹ However, its use as an outbreak investigation tool remains in its infancy. Reports on the use of Facebook during outbreak investigations are limited and concentrate on foodborne illnesses. Kemble et al. reported on the passive detection of a foodborne-associated Group A *Streptococcus* outbreak via multiple posts on a team’s Facebook page,²² while Howland et al. describe their active use of Facebook to administer a foodborne outbreak questionnaire among guests at a wedding reception.²³ In these instances, Facebook provided timely means of publicizing information and gathering accurate data to aid outbreak investigations. As in our experience, it may also prove useful as an adjunctive tool for revealing unreported social networks and gathering places, which could prompt focused re-interviews or expanded contact investigations. Despite the widespread use and timely availability of information on websites such as Facebook, shortcomings include the limited sensitivity and positive predictive value of user-generated content, including self-identified ‘friends’ or acquaintances.²⁴ However, in the context of dwindling public health resources, the use of social media could be considered during investigations not only for TB, but also for outbreaks such as measles and meningitis, where timely administration of post-exposure treatments is required.

Third, detection of a young child with TB is a sensitive marker of recent transmission. As demonstrated with our pediatric index case, routine contact investigations are not always successful in identifying a source case.²⁵ The majority of pediatric TB cases are culture-negative, thereby making prompt identification of the source case imperative for determining the DST profile that informs treatment decisions. However, the need for aggressive attempts at culture confirmation among children suspected to have TB cannot be overstated. Culture confirmation also allows for molecular characterization, which may lead to previously unrecognized source case identification.^{26,27}

Lastly, we devised and implemented an enhanced contact evaluation and treatment protocol based on concerns about ongoing community spread of a PZA-resistant strain with enhanced transmissibility based on the high proportion of contacts, including children, with active disease. While it is suspected that epidemiological factors related to the behavior of certain cases and the baseline health of contacts influenced transmissibility and disease progression, inherent properties of strain virulence cannot be ruled out. This protocol may in part have helped curb the outbreak, as no further epidemiologically linked cases have been detected since 2010.

The retrospective detection of this outbreak provides an opportunity to reflect on the initial challenges and lessons learnt. Based on the standardized naming conventions for TB genotyping, the minor difference in spoligotypes between the 2004 and 2009 genotype clusters prompted different cluster designations.²⁸ This masked the re-emergence of the 2004 strain among the cases identified in 2009. As a result, the outbreak was only first identified based on the unique DST profile. As the outbreak included cases across multiple jurisdictions, swift recognition by individual health departments was not possible. We therefore encourage TB control programs with universal genotyping to consider establishing routine ‘one off’ analyses, in which genotype data are examined secondarily for similarities. Early coordination of genotype investigations at the state level or more broadly may also assist in more timely outbreak identification across jurisdictions.

This report is not without limitations. Despite substantial efforts to obtain complete capture of individuals involved in this outbreak, it is possible that additional exposures or infections remain unidentified. Due to our retrospective use of Facebook, we were not able to confirm mutual contacts in real time. Moreover, only a subset of isolates had all confirmatory testing performed; however, the uniformity of the available results overwhelmingly supports the spread of a clonal strain. Finally, the enhanced contact evaluation and treatment protocol consisted of recommendations to local health departments rather than mandates, precluding complete evaluation.

CONCLUSIONS

In a time when the epidemiology of TB in the United States is concentrated on foreign-born populations, our experiences highlight the need to stay vigilant among all vulnerable populations. This outbreak demonstrates how TB can spread through a population of young adults, many of whom are substance users linked by casual social connections. Although the common PZA_{MR} pattern provided initial clues toward TB spread, the use of genotypic data and the novel incorporation of social media investigations were critical to a clearer understanding of the context of infectivity.

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References

1. Centers for Disease Control and Prevention. Treatment of tuberculosis. MMWR Recomm Rep. 2003; 52(RR-11):1–77.
2. Hannan MM, Desmond EP, Morlock GP, Mazurek GH, Crawford JT. Pyrazinamide-monoresistant *Mycobacterium tuberculosis* in the United States. J Clin Microbiol. 2001; 39:647–650. [PubMed: 11158123]
3. Cheng SJ, Thibert L, Sanchez T, Heifets L, Zhang Y. *pncA* mutations as a major mechanism of pyrazinamide resistance in *Mycobacterium*. Antimicrob Agents Chemother. 2000; 44:528–532. [PubMed: 10681313]

4. Nguyen D, Brassard P, Westley J, et al. Widespread pyrazinamide-resistant *Mycobacterium tuberculosis* family in a low-incidence setting. *J Clin Microbiol.* 2003; 41:2878–2883. [PubMed: 12843016]
5. de Jong BC, Onipede A, Pym AS, et al. Does resistance to pyrazinamide accurately indicate the presence of *Mycobacterium bovis*? *J Clin Microbiol.* 2005; 43:3530–3532. [PubMed: 16000498]
6. Kurbatova EV, Cavanaugh JS, Dalton TS, Click E, Cegielski JP. Epidemiology of pyrazinamide-resistant tuberculosis in the United States, 1999–2009. *Clin Infect Dis.* 2013; 57:1081–1093. [PubMed: 23840002]
7. Werngren J, Sturegard E, Jureen P, Angeby K, Hoffner S, Schon T. Reevaluation of the critical concentration for drug susceptibility testing of *Mycobacterium tuberculosis* against pyrazinamide using wild-type MIC distributions and *pncA* gene sequencing. *Antimicrob Agents Chemother.* 2012; 56:1253–1257. [PubMed: 22203587]
8. Pfyffer GE, Palicova F, Rüsch-Gerdes S. Testing of susceptibility of *Mycobacterium tuberculosis* to pyrazinamide with the nonradiometric BACTEC MGIT 960 system. *J Clin Microbiol.* 2002; 40:1670–1674. [PubMed: 11980940]
9. Chedore P, Bertucci L, Wolfe J, Sharma M, Jamieson F. Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. *J Clin Microbiol.* 2010; 48:300–301. [PubMed: 19923479]
10. Campbell PJ, Morlock GP, Sikes RD, et al. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* 2011; 55:2032–2041. [PubMed: 21300839]
11. National TB Controllers Association, CDC Advisory Group on Tuberculosis Genotyping. Handbook for TB controllers, epidemiologists, laboratorians, and other program staff. Atlanta, GA, USA: US Department of Health and Human Services, CDC; 2004. Guide to the application of genotyping to tuberculosis prevention and control.
12. Thackeray R, Neiger BL, Smith AK, Van Wagenen SB. Adoption and use of social media among public health departments. *BMC Public Health.* 2012; 12:242. [PubMed: 22449137]
13. National Tuberculosis Controllers Association, Centers for Disease Control and Prevention. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. *MMWR Recomm Rep.* 2005; 54(RR-15):1–47.
14. Morlock GP, Crawford JT, Butler WR, et al. Phenotypic characterization of *pncA* mutants of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.* 2000; 44:2291–2295. [PubMed: 10952570]
15. Basinger, E.; Toney, D. TB detection and characterization: the past, the present and the future. Richmond, VA, USA: Division of Consolidated Laboratory Services, Department of General Services, Commonwealth of Virginia; 2009. http://www.vdh.state.va.us/epidemiology/DiseasePrevention/Programs/Tuberculosis/documents/DCLS_labtests.pdf [Accessed February 2014]
16. Virginia Department of Health Division of Disease Prevention. Data security and confidentiality guidelines. Richmond, VA, USA: Virginia Department of Health; 2009. http://www.vdh.virginia.gov/epidemiology/DiseasePrevention/HCS/documents/2013/pdf/SecurityConfidentialityGuidelines11_24_2009.pdf [Accessed February 2014]
17. Virginia Department of Health. TB surveillance reports. Richmond, VA, USA: Virginia DoH; 2011. <http://www.vdh.state.va.us/epidemiology/diseaseprevention/programs/tuberculosis/EpidemiologyandSurveillance.htm> [Accessed February 2014]
18. Sreevatsan S, Pan X, Zhang Y, Kreiswirth BN, Musser JM. Mutations associated with pyrazinamide resistance in *pncA* of *Mycobacterium tuberculosis* complex organisms. *Antimicrob Agents Chemother.* 1997; 41:636–640. [PubMed: 9056006]
19. Zhdanova S, Heyssel SK, Ogarkov O, et al. Primary multidrug-resistant *Mycobacterium tuberculosis* from two regions in Eastern Siberia. *Emerg Infect Dis.* 2013; 19:1649–1652. [PubMed: 24047678]

20. Asghar RJ, Patlan DE, Miner MC, et al. Limited utility of name-based tuberculosis contact investigations among persons using illicit drugs: results of an outbreak investigation. *J Urban Health*. 2009; 86:776–780. [PubMed: 19533366]
21. Eysenbach G. Infodemiology and infoveillance tracking online health information and cyberbehavior for public health. *Am J Prev Med*. 2011; 40 (Suppl 2):S154–S158. [PubMed: 21521589]
22. Kemble SK, Westbrook A, Lynfield R, et al. Foodborne outbreak of group A streptococcus pharyngitis associated with a high school dance team banquet—Minnesota, 2012. *Clin Infect Dis*. 2013; 57:648–654. [PubMed: 23868521]
23. Howland JF, Conover C. Social network as outbreak investigation tool. *Emerg Infect Dis*. 2011; 17:1765–1766. [PubMed: 21888819]
24. Newkirk RW, Bender JB, Hedberg CW. The potential capability of social media as a component of food safety and food terrorism surveillance systems. *Foodborne Pathog Dis*. 2012; 9:120–124. [PubMed: 22217109]
25. Lobato MN, Mohle-Boetani JC, Royce SE. Missed opportunities for preventing tuberculosis among children younger than five years of age. *Pediatrics*. 2000; 106:E75. [PubMed: 11099618]
26. Wootton SH, Gonzalez BE, Pawlak R, et al. Epidemiology of pediatric tuberculosis using traditional and molecular techniques: Houston, Texas. *Pediatrics*. 2005; 116:1141–1147. [PubMed: 16264001]
27. Yeo IK, Tannenbaum T, Scott AN, et al. Contact investigation and genotyping to identify tuberculosis transmission to children. *Pediatr Infect Dis J*. 2006; 25:1037–1043. [PubMed: 17072127]
28. Centers for Disease Control and Prevention, Division of Tuberculosis Elimination. GENType: new genotyping terminology to integrate 24-locus MIRU-VNTR. Atlanta, GA, USA: CDC; 2013. <http://www.cdc.gov/TB/publications/factsheets/statistics/GenotypingTerminology.pdf> [Accessed February 2014]

Figure 1.

Network of outbreak cases: routine contact investigation combined with genotypic cluster investigation and the use of social media investigations allowed for the identification of this network. Pediatric cases are depicted with triangles, adult cases are depicted with circles. Solid lines illustrate direct contact between cases and describe transmission. The star represents a shared reported location, without confirmation of direct contact between cases. The broken lines represent a link between a TB case and a mutual 'friend' identified on Facebook.com. All TB cases shared the same mutual 'friend'. TB = tuberculosis.

Figure 2.

Epidemiology curve of outbreak cases: a total of 30 cases were identified among two linked clusters between December 2004 and August 2010. This image can be viewed online in color at <http://www.ingentaconnect.com/content/iatld/ijtld/2014/00000018/00000005/art00012>

Figure 3.

The proportion of annual pediatric TB cases from this region of central Virginia attributed to this outbreak was 55% for 2009 and 2010. This image can be viewed online in color at <http://www.ingentaconnect.com/content/ijatld/ijatld/2014/00000018/00000005/art00012>

Table 1

Confirmatory laboratory testing results of culture-confirmed cases from each genotype cluster involved in the PZA monoresistance outbreak in Virginia, 2004–2010 ($n = 21$)*

Cluster	2004	2009
Sample size	9	14
PZA resistance, MGIT 960	100% (9/9 tested)	100% (14/14 tested)
<i>pncA</i> mutation	G deletion at 290 (5/5 tested)	G deletion at 290 (8/8 tested)
Spoligotype	677777607760771 (9/9 tested)	677737607760771 (14/14 tested)
MIRU	223226153321 (9/9 tested)	223226153321 (14/14 tested)
MIRU2	143225123224 (8/8 tested)	143225123224 (14/14 tested)
RFLP	Identical 12 bands (9/9 tested)	Identical 12 bands (9/10 tested; 1 had 9 bands)
NTGS cluster designation	VA-028	VA-154
NTGS PCR-type designation	PCR-01046	PCR-05260
Lineage	EuroAmerican	EuroAmerican
Sub-lineage	LAM	LAM

* Spoligotype results differed by one spacer; MIRU and MIRU-2 analyses revealed exact matches; RFLP testing revealed matching patterns on all but one isolate. The differing spoligotypes resulted in the assignment of unique numeric identifiers to each cluster, per the NTGS nomenclature system.

PZA = pyrazinamide; MGIT = Mycobacteria Growth Indicator Tube; MIRU = mycobacterial interspersed repetitive units; RFLP= restriction fragment length polymorphism; NTGS = National Tuberculosis Genotyping Service; PCR = polymerase chain reaction; LAM = Latin American.

Table 2

Clinical and epidemiologic characteristics of TB cases in a pyrazinamide monoresistance outbreak in Virginia, 2004–2010 ($n = 30$)

Characteristics	2004 cluster, culture-confirmed cases ($n = 9$) n (%)	2009 cluster, culture-confirmed cases ($n = 14$) n (%)	2009 cluster, clinical cases ($n = 7$) n (%)
Age, years, median	46	28.5	12
Children aged <15 years	0	1 (7)	5 (71)
Male sex	7 (78)	8 (57)	4 (57)
US-born	7 (78)	14 (100)	7 (100)
African American	5 (56)	13 (93)	7 (100)
Positive TST	6 (67)	7 (50)	7 (100)
Sputum smear-positive for AFB	6 (67)	8 (57)	0
Site of TB disease			
Pulmonary/intra-thoracic	6 (67)	11 (79)	7 (100)
Cavitary, n	2	7	0
Extra-pulmonary	2 (22)	1 (7)	0
Pleural, n	1	1	
Lymph node, n	0	1	
Pericardial, n	1	0	
Both	1 (11)	2 (14)	0
Outcome			
Cured/completed treatment	8 (89)	12 (86)	7 (100)
Died*	0	2 (14)	0
Lost to follow-up	1 (11)	0	0
Reported risk factors			
Contact with infectious TB case	5 (56)	6 (43)	7 (100)
HIV-positive	1 (11)	0	0
Known HCV infection	3 (33)	0	0
Diabetes mellitus	1 (11)	2 (14)	0
End stage renal disease	0	2 (14)	0
Reported substance abuse	5 (56)	6 (43)	1 (14)
History of incarceration	7 (78)	6 (43)	0
Location of transmission			
Jail	2 (22)	1 (7)	0
Workplace	2 (22)	0	0
Home	0	4 (29)	7 (100)
Other social settings	1 (11)	2 (14)	0

* Deaths were unrelated to TB.

TB = tuberculosis; TST = tuberculin skin test; AFB = acid-fast bacilli; HIV = human immunodeficiency virus; HCV = hepatitis C virus.