

identified; 2) this finding adds clues to the identification of potential new co-infections occurring in HIV-infected persons; and 3) this finding underlines the need to investigate the virome content of blood samples in a research context of new microbes as potential threats for transfusion. Further studies aimed at exploring genetic diversity and natural history of gemycircularviruses in human hosts are needed.

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## References

1. Yu X, Li B, Fu Y, Jiang D, Ghabrial SA, Li G, et al. A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proc Natl Acad Sci U S A*. 2010;107:8387–92. <http://dx.doi.org/10.1073/pnas.0913535107>
2. Rosario K, Dayaram A, Marinov M, Ware J, Kraberger S, Staiton D, et al. Diverse circular ssDNA viruses discovered in dragonflies (Odonata: Epiprocta). *J Gen Virol*. 2012;93:2668–81. <http://dx.doi.org/10.1099/vir.0.045948-0>
3. Dayaram A, Opong A, Jaschke A, Hadfield J, Baschiera M, Dobson RC, et al. Molecular characterization of a novel cassava associated circular ssDNA virus. *Virus Res*. 2012;166:130–5. <http://dx.doi.org/10.1016/j.virusres.2012.03.009>
4. Sikorski A, Massaro M, Kraberger S, Young LM, Smalley D, Martin DP, et al. Novel myco-like DNA viruses discovered in the faecal matter of various animals. *Virus Res*. 2013;177:209–16. <http://dx.doi.org/10.1016/j.virusres.2013.08.008>
5. Ng TF, Chen LF, Zhou Y, Shapiro B, Stiller M, Heintzman PD, et al. Preservation of viral genomes in 700-y-old caribou feces from a subarctic ice patch. *Proc Natl Acad Sci U S A*. 2014;111:16842–7. <http://dx.doi.org/10.1073/pnas.1410429111>
6. Kraberger S, Argüello-Astorga GR, Greenfield LG, Galilee C, Law D, Martin DP, et al. Characterisation of a diverse range of circular replication-associated protein encoding DNA viruses recovered from a sewage treatment oxidation pond. *Infect Genet Evol*. 2015;31:73–86. <http://dx.doi.org/10.1016/j.meegid.2015.01.001>
7. Kraberger S, Farkas K, Bernardo P, Booker C, Argüello-Astorga GR, Mesléard F, et al. Identification of novel *Bromus*- and *Trifolium*-associated circular DNA viruses. *Arch Virol*. 2015;160:1303–11. <http://dx.doi.org/10.1007/s00705-015-2358-6>
8. Lamberto I, Gunst K, Müller H, zur Hausen H, de Villiers EM. Mycovirus-like DNA virus sequences from cattle serum and human brain samples from multiple sclerosis patients. *Genome Announc*. 2014;2: pii: e00848–14. <http://dx.doi.org/10.1128/genomeA.00848-14>
9. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U, Fan Ng TF, et al. Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated sewage. *Virology*. 2015;482:98–104. <http://dx.doi.org/10.1016/j.virol.2015.03.011>
10. Biagini P, Touinssi M, Galicher V, de Micco P. KIs virus and blood donors, France. *Emerg Infect Dis*. 2012;18:1374–5. <http://dx.doi.org/10.3201/eid1808.120442>

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## Co-infection with Drug-Susceptible and Reactivated Latent Multidrug-Resistant *Mycobacterium tuberculosis*

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**To the Editor:** Genotyping of *Mycobacterium tuberculosis* (MTB) has identified mixed infections involving >1 MTB strain (1–4), which are clinically relevant when different susceptibility patterns are involved (4–7). We describe a tuberculosis (TB) case-patient with mixed infection in an area of moderate incidence. In a low-resistance setting (monoresistance 4.6%; multiresistance 1.7%), 1 of the strains was drug susceptible and the other was multidrug-resistant (MDR). Molecular fingerprinting and epidemiologic research revealed that the infection corresponded to a recent infection by a susceptible strain and reactivation of an MDR TB strain. The patient was an HIV-negative woman, 47 years of age, who had immigrated to Spain from Romania and had been living in Almería for >3 years. TB was diagnosed in May 2014; she had experienced symptoms for 2 months. Her diagnosis was confirmed 3 years after being studied in Almería as a close contact of her husband, also from Romania, who had tested positive for MDR TB (resistant to rifampin and isoniazid). When she was observed in the contact trace, she tested positive for purified protein derivative, had been vaccinated against the *M. bovis* bacillus Calmette-Guérin strain, and had no radiologic findings or clinical symptoms. Based on the susceptibility profile of her husband, prophylaxis was not prescribed. Her husband adhered to anti-TB treatment for 20 months; all microbiological control test results had been negative since 2 months after starting therapy.

Because her infection was thought to originate from previous contact with an MDR TB case-patient, we assessed her sputum samples for resistance using GenoType-MTBDR-plus (Hain-Lifescience, Nehren, Rhineland-Paltinate, Germany). The test showed hybridization with the same mutant probes (*rpoB*-MUT3; *katG*-MUT1) as those of her husband, the assumed index MDR TB case-patient (Figure, panel A). The pattern was indeterminate because the hybridization for the mutant probes was faint, and intense hybridization was observed for all the *wt* probes (Figure, panel A). Results suggested the simultaneous presence of an MDR strain and a susceptible strain in a respiratory specimen. The presence of the MDR TB strain was confirmed by the phenotypic antibiogram in a BBL MGIT mycobacteria growth indicator tube (Becton Dickinson, Franklin Lakes, NJ, USA) after the isolate had been cultured. GeneXpert (Cepheid, Sunnyvale, CA, USA) was used to analyze 2 respiratory specimens. Results indicated susceptibility to rifampin, revealing the limitations of this test: the use of probes targeting the *wt* sequences failed to detect resistant strains that coexist with a susceptible strain (7).

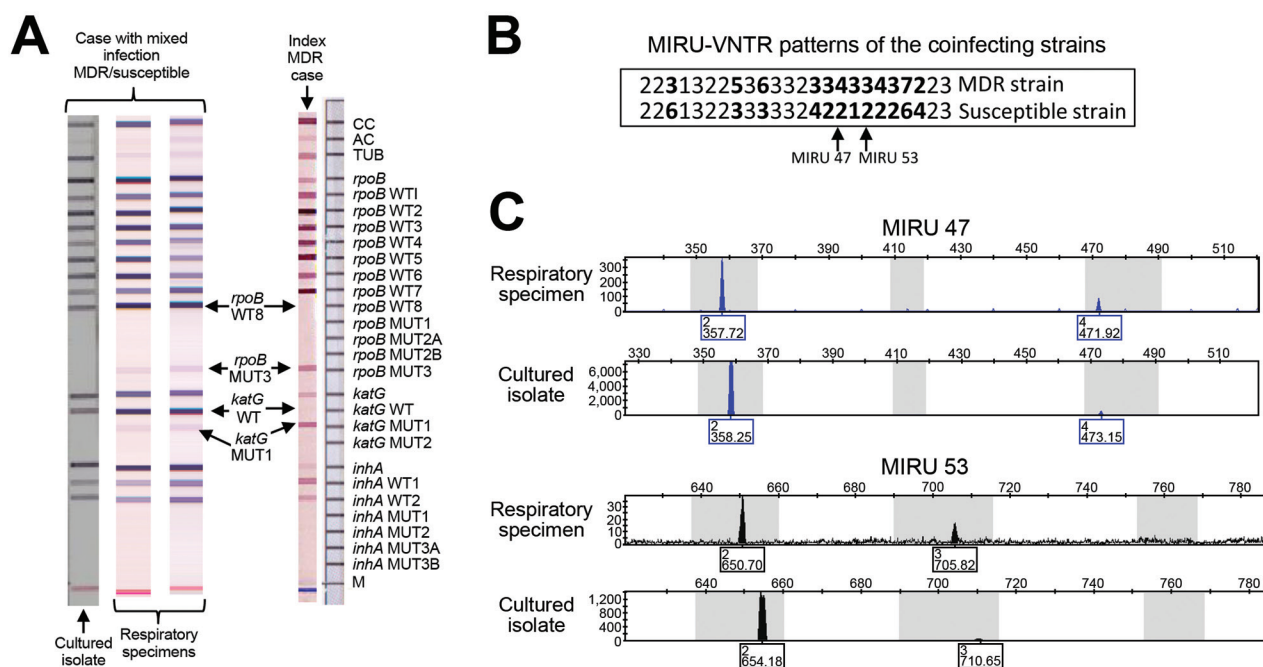
To ascertain the likelihood of 2 co-infecting strains, we analyzed the specimen and the cultured isolate by mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR), which is highly sensitive

for detecting complex infections (8,9). Double alleles were found at 12 loci (Figure, panel B), confirming co-infection.

Peaks in the electropherograms suggested that 1 of the 2 strains was under-represented and its proportion was lower in the cultured isolates (Figure, panel C), indicating that culturing diminished its representation of the minority strain. This finding was consistent with the inability of the GenoType test to detect the MDR strain when applied to the cultured isolate (Figure, panel A). We also detected lower fitness for the MDR strain compared to the susceptible strain ( $p < 0.01$ ) (online Technical Appendix Table, [http://wwwnc.cdc.gov/EID/article/21/11/15-0683\\_Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/21/11/15-0683_Techapp1.pdf)).

Proportions of the resistant and susceptible strains were determined by plating on Middlebrook 7H11 +/- isoniazid (0.4  $\mu\text{L/mL}$ ) and counting single colonies. Because 2% of the colonies were of the resistant strain, we separated the strains and concluded: 1) the strain cultured in the presence of an antimicrobial drug coincided in the population exclusively with the MDR strain from the husband (as shown by MIRU-VNTR); and 2) the co-infecting MDR strain was a minority strain that was under-represented in the cultured isolate.

To analyze the origin of the susceptible strain, we investigated its MIRU-VNTR type in the population-based



**Figure.** Identification of co-infection with drug-susceptible and reactivated latent multidrug-resistant *Mycobacterium tuberculosis* (MDR TB). A) Genotype of multidrug-resistant tuberculosis (MDR TB) and results for the MDR TB index patient and for 2 respiratory specimens and 1 cultured isolate from the case-patient who had a mixed MDR/susceptible infection. The same indeterminate intense-wt/faint mutant pattern test was repeated by using another 2 specimens. B) Mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU-VNTR) types for the 2 strains involved in the co-infection. The results from the analysis yielded double alleles are in bold text. Values for MIRU47 and 53 are indicated. C) Selection of 2 electropherograms representative of 2 (MIRU47 and 53) of the 12 loci with double alleles caused by mixed infection. Data that were obtained from direct analysis of a respiratory specimen or from a cultured isolate are shown.

molecular epidemiology survey (10) and found another 4 cases (from 2008, 2011 [2 cases], and 2014). Three case-patients had emigrated from Romania, and all 5 case-patients lived in the same area of Almeria. These data indicated that the susceptible strain was circulating in the geographic/epidemiological context of the current case-patient before and when she tested positive for that strain; therefore, she likely acquired the susceptible strain through recent transmission.

The presence of susceptible and resistant strains in a patient should be considered even in moderate incidence settings and where resistance rates are not high. Underdetection of these cases could lead to misinterpretation when MDR became apparent after treatment of susceptible strains. Diagnostic laboratories could easily screen for mixed infections by applying MIRU-VNTR. However, only by integrating clonal analysis, refined molecular typing, and epidemiologic data from universal genotyping programs can we clarify the reasons underlying complex MTB infections. For this case-patient, a recent infection with a susceptible strain coincided with or could have triggered reactivation of a latent infection involving an MDR strain acquired through close contact years previously. We emphasize the alteration of the true clonal complexity of an infection induced by culturing specimens and that some commercial tests do not identify complex MTB infections. These findings are particularly relevant when the infection involves resistant strains such as those found in this case-patient.

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### References

- Warren RM, Victor TC, Streicher EM, Richardson M, Beyers N, Gey van Pittius NC, et al. Patients with active tuberculosis often have different strains in the same sputum specimen. *Am J Respir Crit Care Med*. 2004;169:610–4. <http://dx.doi.org/10.1164/rccm.200305-714OC>
- Shin SS, Modongo C, Ncube R, Sepako E, Klausner JD, Zetola NM. Advanced immune suppression is associated with increased prevalence of mixed-strain *Mycobacterium tuberculosis* infections among persons at high risk for drug-resistant tuberculosis in Botswana. *J Infect Dis*. 2015;211:347–51. <http://dx.doi.org/10.1093/infdis/jiu421>
- Dickman KR, Nabyonga L, Kateete DP, Katabazi FA, Asiimwe BB, Mayanja HK, et al. Detection of multiple strains of *Mycobacterium tuberculosis* using MIRU-VNTR in patients with pulmonary tuberculosis in Kampala, Uganda. *BMC Infect Dis*. 2010;10:349. <http://dx.doi.org/10.1186/1471-2334-10-349>
- Niemann S, Richter E, Rüscher-Gerdes S, Schlaak M, Greinert U. Double infection with a resistant and a multidrug-resistant strain of *Mycobacterium tuberculosis*. *Emerg Infect Dis*. 2000;6:548–51. <http://dx.doi.org/10.3201/eid0605.000518>
- Hingley-Wilson SM, Casey R, Connell D, Bremang S, Evans JT, Hawkey PM, et al. Undetected multidrug-resistant tuberculosis amplified by first-line therapy in mixed infection. *Emerg Infect Dis*. 2013;19:1138–41. <http://dx.doi.org/10.3201/eid1907.130313>
- Mendez MP, Landon ME, McCloud MK, Davidson P, Christensen PJ. Co-infection with pansensitive and multidrug-resistant strains of *Mycobacterium tuberculosis*. *Emerg Infect Dis*. 2009;15:578–80. <http://dx.doi.org/10.3201/eid1504.080592>
- Zetola NM, Shin SS, Tumedji KA, Moeti K, Ncube R, Nicol M, et al. Mixed *Mycobacterium tuberculosis* complex infections and false-negative results for rifampin resistance by GeneXpert MTB/RIF are associated with poor clinical outcomes. *J Clin Microbiol*. 2014;52:2422–9. <http://dx.doi.org/10.1128/JCM.02489-13>
- García de Viedma D, Alonso Rodríguez N, Andrés S, Ruiz Serrano MJ, Bouza E. Characterization of clonal complexity in tuberculosis by mycobacterial interspersed repetitive unit-variable-number tandem repeat typing. *J Clin Microbiol*. 2005;43:5660–4. <http://dx.doi.org/10.1128/JCM.43.11.5660-5664.2005>
- Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D, Locht C. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. *J Clin Microbiol*. 2001;39:3563–71. <http://dx.doi.org/10.1128/JCM.39.10.3563-3571.2001>
- Alonso-Rodríguez N, Martínez-Lirio M, Sánchez ML, Herranz M, Penafiel T, Bonillo Mdel C, et al. Prospective universal application of mycobacterial interspersed repetitive-unit-variable-number tandem-repeat genotyping to characterize *Mycobacterium tuberculosis* isolates for fast identification of clustered and orphan cases. *J Clin Microbiol*. 2009;47:2026–32. <http://dx.doi.org/10.1128/JCM.02308-08>

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## Sensitivity to Polymyxin B in El Tor *Vibrio cholerae* O1 Strain, Kolkata, India

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**To the Editor:** The epidemiology of cholera, especially in Africa and Asia, has periodically changed in subtle ways (1). The recent cholera epidemic in Haiti, a Caribbean country with no cholera cases in decades, affected >500,000 persons, caused ≈8,000 deaths, and brought this illness to the forefront of Haitian public health concerns

# Co-Infection with Drug-Susceptible and Reactivated Latent Multidrug-Resistant *Mycobacterium tuberculosis*

## Technical Appendix

**Technical Appendix Table.** Fitness analysis, assayed in vitro\*, for 2 co-infecting strains of drug-susceptible and reactivated latent multidrug-resistant *Mycobacterium tuberculosis*†

| Strains            | Lag phase (hours to positive threshold [75 GU]) | Growth rate (hours for the 3000–4000 GU increase) |
|--------------------|---|---|
| Susceptible strain | 220.17 ± 14.85                                  | 28 ± 1.13   |
| MDR strain         | 289.67 ± 6.12                                   | 46.4 ± 2.26                                       |

\*MGIT960 culture system, Becton Dickinson, Franklin Lakes, NJ, USA.

†Mean ± SD values were calculated for data from 3 independent experiments. GU, growth units.