# THE LANCET Infectious Diseases

## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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### Supplementary appendix

Schwartz NG, Hernandez-Romieu AC, Annambhotla P, et al. Nationwide tuberculosis outbreak in the USA linked to a bone graft product: an outbreak report.

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#### **Supplementary methods**

Real-time polymerase chain reaction (rt-PCR) and culture

Approximately 100 mg of the bone allograft material was transferred to a screw-top tube containing 400  $\mu$ L 1x TE with 100  $\mu$ L 1.0 mm and 200  $\mu$ L 0.1 mm glass beads along with an extraction control. The sample was heat inactivated and then homogenized using a BioSpec Beadbeater as recommended by the manufacturer. Deoxyribonucleic acid (DNA) was isolated, and the presence of the *M. tuberculosis* complex DNA was detected using a real-time polymerase chain reaction (PCR) assay that amplifies the IS *1081* and IS6110 insertion elements. The remaining material was homogenized in 7 mL sterile phosphate-buffered saline (PBS) using a gentleMacs Tissue Homogenizer. The soluble portions of the samples were centrifuged to pellet any potential mycobacteria in the sample. The supernatant was decanted, and the pellet resuspended in 3 mL sterile PBS. 400  $\mu$ L was used to inoculate BD BACTEC Mycobacterial Growth Indicator Tube (MGIT) media in duplicate. 1.5 mL of 800  $\mu$ g/mL erythromycin was added to the MGIT PANTA dry mix supplied in the BACTEC MGIT Supplement Kit. Then 15 mL of the reconstituting fluid was added to the PANTA/erythromycin mix. 0.8 mL of the reconstituted PANTA/erythromycin mix was added to each MGIT tube. Using a swab, commercially available solid media (Stonebrinks, Lowenstein-Jensen, and Mycobactosel-Lowenstein-Jensen) and in-house prepared solid media (7H11 with pyruvate and 7H11 with glycerol) were inoculated.

#### Whole-genome sequencing and phylogenetic analysis

Nextera XT sequencing libraries were sequenced (2 x 150 bp reads) on the NextSeq 500 platform using NextSeq 500/550 Mid Output Kit v2.5 300 cycle chemistry. Samples with a sequence read set Q30 frequency <85% were re-sequenced. Reference-guided assemblies were created using BioNumerics 7.6 Reference Mapper v 1.2.3 using *M. tuberculosis* strain H37Rv (NC00962.3) as the reference with the following settings for base calling: minimum total coverage=3, minimum forward coverage=1, minimum reverse coverage=1, single base threshold=0.75, double base threshold=0.85, triple base threshold=0.95, gap threshold=0.5. A bowtie-based algorithm was used.¹ Samples with an average genome coverage <25 were re-sequenced. Reference-guided assembly samples were compared using BioNumerics 7.6 single nucleotide polymorphism (SNP) analysis filters. For a SNP to be retained in the comparison, the base in all samples must have had a total coverage of 5 reads, must not have contained ambiguous bases, must not have contained unreliable bases, must not have contained gaps, and must not have been within 12 base-pairs of another SNP. SNPs that were non-informative (identical in all samples) were also excluded. The comparison was visualized using a phylogenetic tree constructed using the neighbor-joining method. *M. tuberculosis* strain H37Rv was used as the outgroup and the phylogenetic tree was rooted at the midpoint of the longest branch.

#### Molecular Detection of Drug Resistance (MDDR)

CDC's National Tuberculosis Reference Laboratory performed Sanger DNA sequencing to assess an *M. tuberculosis* isolate from unused bone allograft material for genetic mutations associated with resistance to rifampin, isoniazid, ethambutol, pyrazinamide, fluoroquinolones, kanamycin, capreomycin, and amikacin. Loci were sequenced in the following genes: rpoB (associated with rifampin resistance); inhA, katG, and fabG1 (associated with isoniazid resistance); embB (associated with ethambutol resistance); pncA (associated with pyrazinamide resistance); gyrA and gyrB (associated with fluoroquinolone resistance); rrs (associated with resistance to kanamycin, amikacin, and capreomycin), tlyA (associated with capreomycin resistance), and eis (associated with kanamycin resistance). Detailed methods and performance characteristics are described in a previously published, publicly available guide.<sup>2</sup>

#### Growth-based drug susceptibility testing

CDC's National Tuberculosis Reference Laboratory used an indirect agar proportion method (Middlebrook 7H10 agar) to test an *M. tuberculosis* isolate from unused bone allograft material for susceptibility to 12 drugs: isoniazid, rifampin, ethambutol, ciprofloxacin, ofloxacin, streptomycin, kanamycin, capreomycin, amikacin, rifabutin, ethionamide, and para-aminosalicylic acid. Resistance was defined as >1% growth on drug-containing medium compared to drug-free medium. Pyrazinamide susceptibility was tested using the BD BACTEC MGIT 960 system.

#### **Supplementary tables**

Table S1. Evidence of surgical site tuberculosis disease in bone allograft recipients

	No.	No. with available data	Percentage of all 113 recipients	Percentage of recipients with available data
Microbiologic or imaging evidence	83	106	73%	78%
Microbiologic evidence	54	63	48%	86%
Positive AFB smear	40	61	35%	66%
Positive NAAT	31	44	27%	70%
Positive culture	51	58	45%	88%
Imaging evidence	80	106	71%	75%
Abscess or fluid collection	69	106	61%	65%
Osteomyelitis	36	106	32%	34%
Discitis	18	106	16%	17%
Meningeal enhancement (without clinical signs or symptoms of meningitis)	2	106	2%	2%
Other abnormality compatible with tuberculosis	3	106	3%	3%

AFB=acid-fast bacilli. NAAT=nucleic acid amplification test.

Table S2. Evidence of pulmonary tuberculosis disease in bone allograft recipients

	No.	No. with available data	Percentage of all 113 recipients	Percentage of recipients with available data
Microbiologic or imaging evidence	27	110	24%	25%
Microbiologic evidence	13	58	12%	22%
Positive AFB smear	4	58	4%	7%
Positive NAAT	11	51	10%	22%
Positive culture	11	56	10%	20%
Imaging evidence	23	110	20%	21%
Miliary lesions	13	110	12%	12%
Multifocal nodular lesions	19	110	17%	17%
Cavitary lesions	3	110	3%	3%

AFB=acid-fast bacilli. NAAT=nucleic acid amplification test.

Table S3. Evidence of central nervous system tuberculosis disease in bone allograft recipients

	No.	No. with available data	Percentage of all 113 recipients	Percentage of recipients with available data
Microbiologic or imaging evidence	3*	10	3%	30%
Microbiologic evidence	1	5	1%	20%
Positive AFB smear	0	5	0%	0%
Positive NAAT	1	3	1%	33%
Positive culture	1	3	1%	33%
Imaging evidence	3	8	3%	38%
Brain tuberculomas	2	8	2%	25%
Leptomeningeal enhancement of the cervical spine (with clinical signs and symptoms of meningitis)	1	8	1%	13%

AFB=acid-fast bacilli. NAAT=nucleic acid amplification test.

<sup>\*</sup> One additional patient had clinically diagnosed tuberculous meningitis based on cerebrospinal fluid cell counts and chemistries.

Table S4. Evidence of tuberculosis disease at other anatomic sites in bone allograft recipients

	No.	No. with available data*	Percentage of all 113 recipients	Percentage of recipients with available data*
Positive blood culture	3	5	3%	
Positive culture of liver specimen	1	2	1%	
Positive culture of bone marrow specimen	1	2	1%	

<sup>\*</sup>For the anatomic sites in this table, negative test results were not systematically reported so percentages among recipients with available data were not calculated.

#### **Supplementary references**

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