

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kennedy BS, Bedard B, Younge M, et al. Outbreak of *Mycobacterium chelonae* infection associated with tattoo ink. N Engl J Med 2012;367:1020-4. DOI: 10.1056/NEJMoa1205114

Supplementary Materials

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Outbreak of *Mycobacterium chelonae* Infection Associated with Tattoo Ink

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FIGURE S1. Image of Histology and Acid-Fast Bacilli (AFB) Stain from Tattooed Lesions.

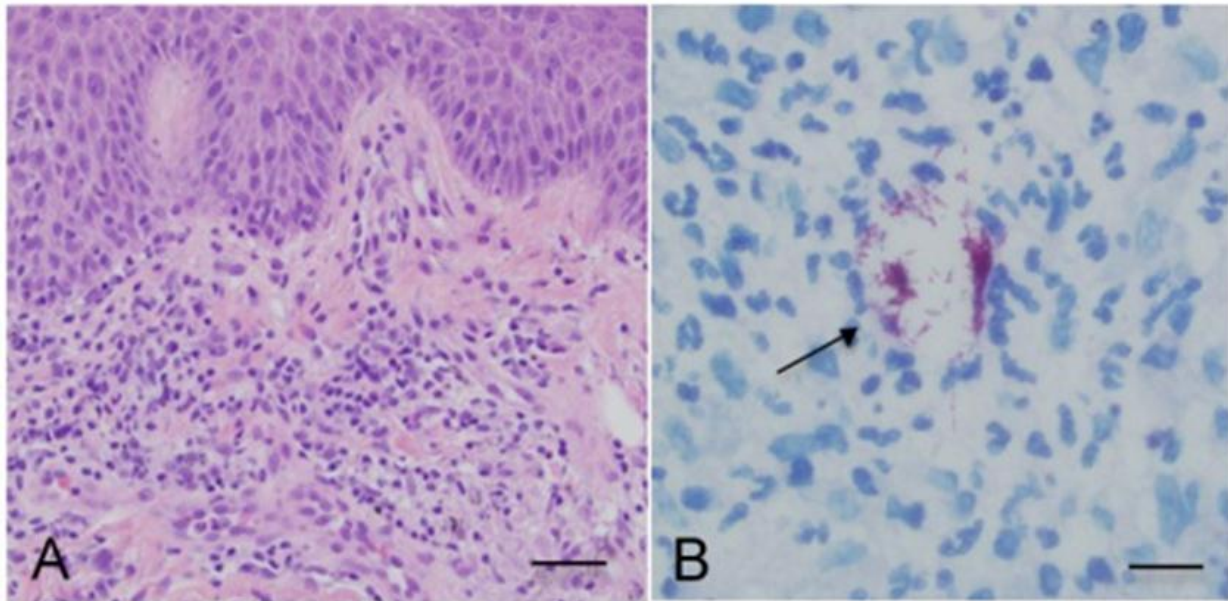
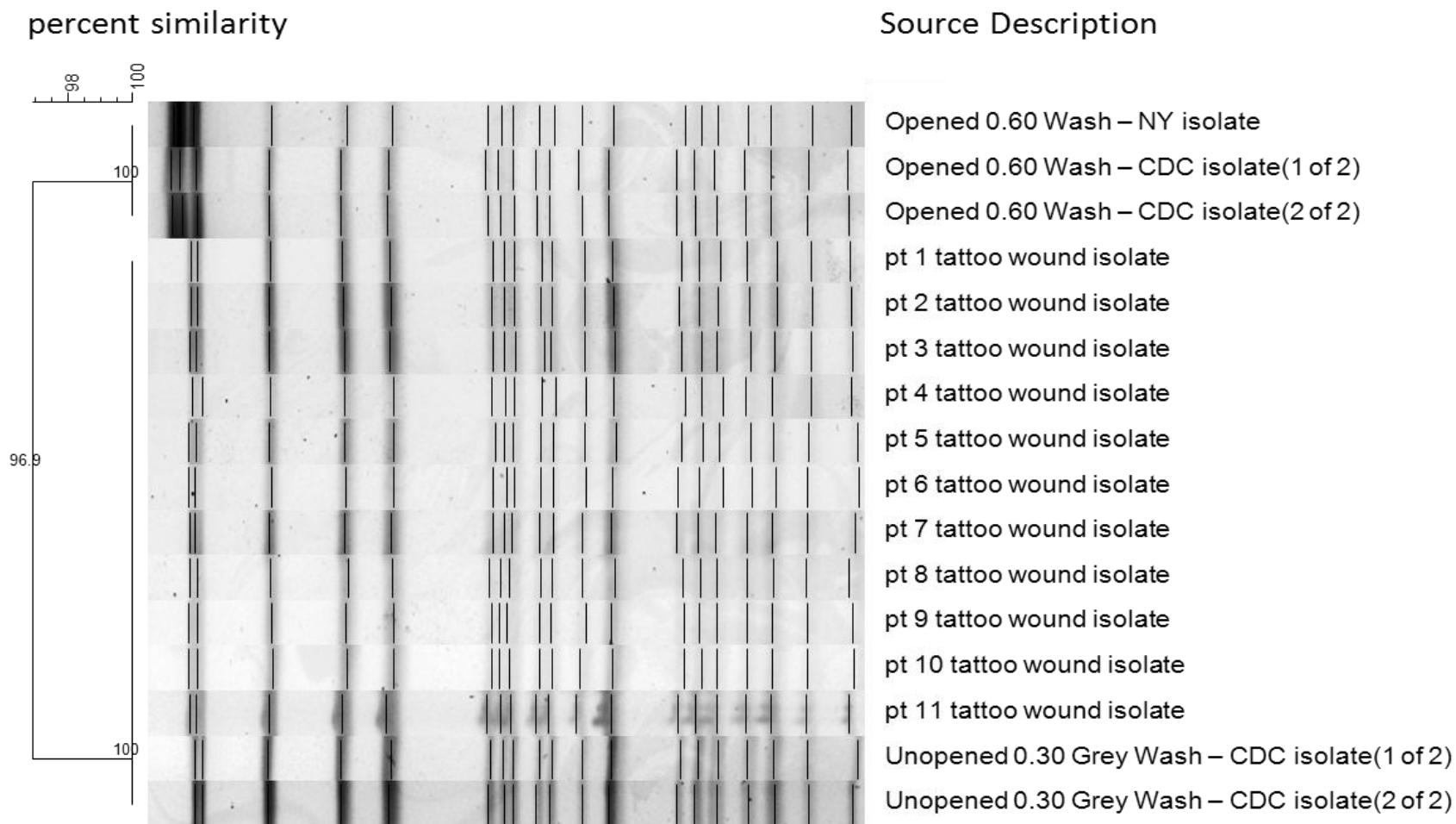


Figure S1: A) Hematoxylin and eosin stained section of a patient shows a sparse infiltrate of lymphocytes and histiocytes in the upper dermis. Granulomas were not identified. Bar~ 40 μ M. B) Ziehl-Neelsen acid fast stain from a patient shows a collection (arrow) of acid-fast organisms consistent with atypical mycobacteria. Bar~15 μ M.

FIGURE S2. Pulse-Field Gel Electrophoresis (PFGE): Genetic Relatedness of *M. chelonae* from Tattoo Wash and Patients.



Pt 1=Case 6; Pt 2=Case 10; Pt 3=Case 8; Pt 4=Case 4; Pt 5=Case 7; Pt 6=Case 15; Pt 7=Case 18; Pt 8=Case 3; Pt 9=Case 11; Pt 10=Case 13; Pt 11=Case 2; Pt 12 (not shown, negative culture result)=Case 1. For the opened 0.60 Wash, all three isolates came from the same bottle; for the unopened 0.30 Grey Wash, both isolates came from the same bottle. An unopened bottle of 0.60 Grey Wash and an unopened bottle of 0.90 Grey Wash were also tested and had negative culture results for *M. chelonae* (not shown).

TABLE S1. Detailed Summary of Cases.

Case Number	Case Definition	Month of Tattoo	Month of Rash	Results
1	Probable	September	October	Rash on arm; Bx= granuloma; Cx= negative <i>M. chelonae</i> (delayed specimen processing time); PFGE= Not done; Tx= gentamicin cream then azithromycin and doxycycline day 22
2	Confirmed	October	October	Rash on left arm; Bx= granuloma; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin then doxycycline day 34
3	Confirmed	November	November	Rash right arm; Bx= granuloma; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= azithromycin and doxycycline
4	Confirmed	November	November	Rash on rib cage; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
5	Probable	November	November	Rash on right arm; Bx= sparse lymphohistiocytic infiltrate; Cx= negative <i>M. chelonae</i> (pre-treated with 250 mg clarithromycin); PFGE= Not done; Tx= clarithromycin 500 mg twice daily
6	Confirmed	November	November	Rash on left arm; Bx= sparse lymphohistiocytic infiltrate with multinucleated giant cells; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
7	Confirmed	November	November	Rash on wrist; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
8	Confirmed	November	November	Rash on right arm; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= azithromycin
9	Probable	November	November	Rash left leg; Bx= Not confirmed (done in California); Cx= Not done; PFGE= Not done; Tx= clarithromycin
10	Confirmed	November	November	Rash right arm; Bx= granuloma; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
11	Confirmed	November	November	Rash right arm; Bx= granuloma; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
12	Confirmed	November	November	Rash on right arm; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= Not done; Tx= azithromycin then doxycycline day 13

13	Confirmed	November	November	Rash on right arm; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
14	Suspected	November	November	Rash on arm; Bx= Not done; Cx= Not done; PFGE= Not done; Tx= Not treated (patient declined)
15	Confirmed	November	December	Rash on back; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= azithromycin
16	Probable	November	December	Rash on foot; Bx= sparse lymphohistiocytic infiltrate; Cx= negative <i>M. chelonae</i> (early laboratory learning curve); PFGE= Not done; Tx= azithromycin then doxycycline day 28
17	Confirmed	November	December	Rash on shoulder; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= Not done; Tx= azithromycin and doxycycline
18	Confirmed	December	December	Rash on arm; Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= indistinguishable pattern; Tx= clarithromycin
19	Confirmed	Unknown	October	Rash on left arm (uncertainty: tattoo occurred Sep - Oct and rash began Oct); Bx= sparse lymphohistiocytic infiltrate; Cx= positive <i>M. chelonae</i> ; PFGE= Not done; Tx= clarithromycin and doxycycline

Bx=biopsy; Cx=culture; Tx=treatment; PFGE=pulse-field gel electrophoresis.

Suspected case: anyone receiving a tattoo with the premixed grey ink between May and December 2011; subsequently developing a persistent, raised, erythematous rash within one to three weeks of tattoo and confined to areas tattooed with the premixed grey ink.

Probable case: all the above plus either a tissue biopsy specimen positive for acid-fast bacilli (AFB) or histopathology showing evidence of a granulomatous reaction or lymphohistiocytic infiltrate; or showing clinical improvement after initiating appropriate antimicrobial treatment for presumed *M. chelonae*.

Confirmed case: all the above plus laboratory-confirmed identification of *M. chelonae*.

