Toxigenic *Corynebacterium diphtheriae* Infection in Cat, Texas, USA

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We report a toxigenic strain of *Corynebacterium diphtheriae* isolated from an oozing dermal wound in a pet cat in Texas, USA. We also describe the epidemiologic public health efforts conducted to identify potential sources of infection and mitigate its spread and the molecular and genetic studies performed to identify the bacterium.

Diphtheria, caused by toxigenic strains of the bacterium *Corynebacterium diphtheriae*, can result in life-threatening respiratory disease or cutaneous infections. Toxigenicity is contingent on successful bacterial expression of diphtheria toxin, encoded by a toxin gene (tox). Toxigenic *C. diphtheriae* is considered nearly exclusively a human pathogen, and humans are believed to be the reservoir. Because of high population coverage with diphtheria toxoid–containing vaccines, few diphtheria cases are reported in the United States. The most recently reported toxigenic infections were cutaneous and associated with international travel (*1*–*4*).

A 2016 article reviewing available literature on *C. diphtheriae* isolated from animals identified 12 cases globally, 4 in dogs, 4 in cats, 2 in horses, 1 in a cow, and 1 in a fox. These infections were toxigenic only in 2 dogs and the 2 horses; 1 of the horses was identified in the United States (*5*,*6*). In contrast, toxigenic *Corynebacterium ulcerans* is a zoonotic organism that causes diphtheria-like illness in humans clinically indistinguishable from illness caused by toxigenic *C. diphtheriae*; it is more common than the diphtheria pathogen among household pets and their owners (*7*).

To date, toxigenic diphtheria has not been detected in cats; however, nontoxigenic strains have been identified, including 2 from the ears of cats in the United States and 1 from the nose of a cat in Belgium (*8*,*9*). Although these 3 strains contained the tox gene, they were not toxin producing. Of note, the strains identified in the United States have recently been reclassified as a novel species, *C. rouxii*, because of biochemical and genetic differences with *C. diphtheriae* (*10*).

Recommended public health response to toxigenic diphtheria infections in humans in the United States involves isolating and treating the index case-patient, identifying contacts, and vaccinating the patient and contacts with diphtheria toxoid–containing vaccine if it has been ≥5 years since the last dose (*11*). After treatment is completed, the index case-patient should be tested to confirm eradication of toxigenic *C. diphtheriae* and contacts monitored for development of diphtheria illness for 7–10 days after their most recent exposure; nasal and throat swab specimens should be collected to test for carriage, and prophylactic antibiotics should be administered. No formal recommendations exist for toxigenic diphtheria in animals because of its rarity, but health departments may pursue interventions similar to those to prevent transmission in humans.

In October 2020, a veterinary clinic in southern Texas, USA, evaluated a male domestic shorthair cat 10 years of age for an oozing wound with multiple abscess pockets in its left flank. The clinic reported culturing *Mycobacterial farcinogenes* from a similar lesion on the cat in May 2018. A swab of the new wound was submitted for culturing to the Texas A&M Veterinary

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Medical Diagnostic Laboratory (College Station, TX, USA). The cat was empirically started on marbofloxacin, but after the owner reported worsening of the wound 2 days later, the attending veterinarian added amoxicillin/clavulanic acid to the regimen. The laboratory isolated 2 bacteria, *C. diphtheriae* and *M. farcinogenes*, and sent the *C. diphtheriae* isolate to the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) for confirmation and toxigenicity testing. An investigation was conducted to identify possible exposures to toxigenic *C. diphtheriae*, identify potential human and animal carriers, and provide prevention measures. We report details of the investigation and subsequent molecular and genetic studies.

The Study

The owner of the index cat lived in a house with her husband and reported having no regular visitors the month before the cat developed the flank abscess. The owner reported they had 5 indoor-only cats, including the index cat, and 4 dogs that spent time both indoors and outdoors. The cat's owner had received tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine 3 years earlier; her husband provided no vaccine history. Cameron County Public Health (San Benito, TX, USA) collected an oropharyngeal swab specimen from the owner in November 2020 and submitted it to the Texas Department of State Health Services laboratory (Harlingen, TX, USA) for testing; the sample was negative for *C. diphtheriae*. Her husband did not submit a specimen. Both refused antibiotic prophylaxis, and the husband refused diphtheria toxoid–containing vaccine.

The owners allowed oropharyngeal swab specimens to be collected from the 4 contact cats but refused to have their dogs tested. The cat samples were submitted to CDC but were negative for *C. diphtheriae* by isolation or PCR detection. One posttreatment swab specimen collected from the wound of the index cat in November was negative for *C. diphtheriae*. In December, the owner reported the wound appeared to be healing.

Ten veterinary staff were identified as having potential exposures to the *C. diphtheriae* wound; 9 worked with the abscess wearing gloves and masks but no eye protection, and 1 was bitten while handling the cat. The Cameron County Public Health clinic collected oropharyngeal swab specimens from 9/10 exposed staff, and all tested negative for *C. diphtheriae* at the Texas Department of State Health Services laboratory. Six of 10 exposed staff received prophylactic antibiotics; 5/10 reported receiving no diphtheria toxoid vaccine within 5 years and so received vaccine boosters, and the remaining 5 reported having received diphtheria toxoid vaccine within 5 years. Human and animal contacts were assessed for clinical signs and symptoms, including skin lesions, consistent with diphtheria, but no signs or symptoms were observed.

CDC conducted microbiologic and molecular characterization of the *C. diphtheriae* isolate (named PC1297), as described elsewhere (*12*,*13*). The isolate was confirmed as *C. diphtheriae* biotype gravis, and PCR confirmed presence of the tox gene. Modified Elek testing showed the isolate produced diphtheria toxin (*14*). The isolate was further characterized by whole-genome shotgun sequencing on an Illumina Miseq (https://www.illumina.com) (Appendix, https://wwwnc.cdc.gov/EID/article/28/8/22-0018-App1.pdf). Genome sequence-based multilocus sequence typing identified the isolate as ST705, unique among the 754 publicly available *C. diphtheriae* isolate sequences, and genome assembly confirmed presence of tox-encoding corynephage (Appendix) (*15*). Phylogenetic reconstruction of 273 *C. diphtheriae* isolate sequences, representing 270 unique sequence types and including 8 isolates from domestic animals. The results indicated that PC1297 was not related to isolates from previous cases reported in cats, including those now classified as *C. rouxii* (Appendix), nor was it closely related to any available human sequences; the nearest neighboring sequence in the phylogenic tree, ERR3932636, sequence type 669, was 6,948 single-nucleotide polymorphisms distant.

Conclusion

We report public health response to a rare case of cutaneous toxigenic diphtheria in a pet cat. Not all animal and human contacts could be tested, but *C. diphtheriae* was not detected among those tested; no source for the infection was identified. Comparative genomic analyses suggested that the identified strain differed from publicly available sequences of *C. diphtheriae*, including those from domestic pets, and the strain was not related to the neighboring *C. rouxii* sp. nov. Because of the limited availability of *C. diphtheriae* sequences from animals, there was insufficient data to determine whether the source of infection was from human or animal contact. Whereas our findings do not confirm whether animals might serve as reservoirs for diphtheria, they highlight the need for further study regarding transmission and environmental health. This case also reiterates the criticality of promptly discovering and identifying *C. diphtheriae* infections in companion animals for preventing spread of the disease to susceptible animals and humans.

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About the Author

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Appendix

Methods

C. diphtheriae PC1297 was grown on trypticase soy agar with 5% sheep blood at 37 °C for 24 h. Genomic DNA was extracted using the Maxwell RSC whole Blood DNA kit (Promega, https://www.promega.com) and the concentration was determined using the Qubit dsDNA broad range quantification kit (Thermo Fisher Scientific, https://www.thermofisher.com). Paired-end libraries (2 X 250bp) were prepared with the NEBNext Ultra DNA Library Prep Kit (New England Biolabs, https://www.neb.com) and sequenced on a Miseq using reagent v2, 500-cycle kit (Illumina, https://www.illumina.com). Raw Illumina read quality was assessed using FastQC v0.11.5 (*1*) before trimming and filtering with BBDuk (*2*) and Cutadapt (*3*). Trimmed reads were de novo assembled using SPAdes v3.9 (*4*) and evaluated with QUAST v4.5 (*5*).

Genome sequence-based molecular typing by multi-locus sequence typing (MLST) was determined from the assembled contigs according to stringMLST (*6*), and further confirmed with trimmed sequencing reads using SRST2 (*7*). The presence of diphtheria toxin homologs was determined by tBLASTn query of the assembled genomes using select references: Corynephage beta A and B subunits (NCBI accession no. P00588), Corynephage omega beta Diphtheria toxin (NCBI accession no. P00587), and Corynephage beta Diphtheria toxin homolog (NCBI accession no. P00589).

Publicly available *C. diphtheriae* genomic data for 273 isolates representing 270 unique sequence types were retrieved from the NCBI Sequence Read Archive (SRA) for phylogenetic comparison (Appendix Table 1). Raw reads were trimmed and filtered as described above. SNPs were determined by mapping all trimmed reads to the reference genome of NCTC13129 (NC_002935) using Snippy v4.3.8 with default settings (*8*). The phylogeny was reconstructed

from the resulting core SNP alignment using maximum-likelihood with RaxML v8.2.9 (*9*) and the tree was visualized with iToL v6 (*10*) (Appendix Figure 1).

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Appendix Table. *C. diphtheriae* isolate sequences used for phylogenetic analysis*

ID	atpA	dnaE	dnaK	fusA	leuA	odhA	rpoB	ST	Species (animal)†
ERR2094322	2	1	4	1	3	3	5	97	*C. diphtheriae*
ERR2094324	2	26	55	29	3	3	2	184	*C. diphtheriae*
ERR2094326	3	2	4	30	3	3	2	251	*C. diphtheriae*
ERR2094331	28	1	20	19	3	16	20	102	*C. diphtheriae*
ERR2094333	30	23	63	25	3	3	28	217	*C. diphtheriae*
ERR2094334	4	11	13	7	3	56	27	432	*C. diphtheriae*
ERR2094336	28	2	60	30	3	3	3	367	*C. diphtheriae*
ERR2094337	2	1	20	19	42	16	31	412	*C. diphtheriae*
ERR2167138	2	1	20	16	3	3	5	83	*C. diphtheriae*
ERR2173761	4	1	102	4	5	5	35	503	*C. diphtheriae*
ERR2173765	2	51	9	60	8	3	2	486	*C. diphtheriae*
ERR2173766	30	1	93	25	3	3	6	398	*C. diphtheriae*
ERR2173767	4	46	4	1	5	3	2	473	*C. diphtheriae*
ERR2173768	22	4	60	62	3	1	4	505	*C. diphtheriae*
ERR2173771	4	10	3	1	7	3	13	469	*C. diphtheriae*
ERR2173772	3	53	32	19	3	4	4	506	*C. diphtheriae*
ERR2173773	2	2	60	2	3	3	2	474	*C. diphtheriae*
ERR2173774	30	10	102	4	3	3	35	502	*C. diphtheriae*
ERR2217012	2	3	3	2	7	3	3	406	*C. diphtheriae*
ERR2217013	2	2	9	25	3	3	2	302	*C. diphtheriae*
ERR2217014	1	1	88	1	29	52	4	379	*C. diphtheriae*
ERR2217016	4	21	29	1	3	3	3	120	*C. diphtheriae*
ERR2217017	13	4	8	44	3	3	13	433	*C. diphtheriae*
ERR3331350	16	4	8	1	2	30	9	151	*C. diphtheriae*
ERR3331352	3	2	4	19	3	16	9	455	*C. diphtheriae*
ERR3331354	5	2	7	1	3	5	8	10	*C. diphtheriae*
ERR3331355	3	2	3	6	3	3	2	67	*C. diphtheriae*
ERR3331356	16	8	50	1	2	30	9	161	*C. diphtheriae*
ERR3331365	3	3	4	2	3	3	3	209	*C. diphtheriae*
ERR3932476	2	1	33	28	11	2	2	82	*C. diphtheriae*
ERR3932480	2	1	28	4	39	4	9	222	*C. diphtheriae*
ERR3932481	2	1	32	19	13	3	6	80	*C. diphtheriae*
ERR3932482	2	10	17	13	40	2	4	223	*C. diphtheriae*
ERR3932483	35	10	33	19	13	3	6	192	*C. diphtheriae*
ERR3932485	2	4	3	1	7	37	4	225	*C. diphtheriae*
ERR3932487	13	29	66	44	1	1	6	227	*C. diphtheriae*
ERR3932488	2	1	2	1	7	3	9	233	*C. diphtheriae*
ERR3932491	22	18	6	1	3	1	4	116	*C. diphtheriae*
ERR3932492	5	6	7	6	6	3	8	25	*C. diphtheriae*
ERR3932495	3	1	16	4	13	3	5	146	*C. diphtheriae*
ERR3932496	2	9	4	1	30	28	2	131	*C. diphtheriae*
ERR3932497	2	3	3	19	13	3	6	132	*C. diphtheriae*
ERR3932498	3	2	5	1	27	5	9	150	*C. diphtheriae*
ERR3932499	33	15	4	1	3	3	2	140	*C. diphtheriae*
ERR3932500	1	2	9	7	8	3	2	183	*C. diphtheriae*
ERR3932502	2	1	45	1	5	3	9	123	*C. diphtheriae*
ERR3932504	3	2	4	3	4	4	4	4	*C. diphtheriae*
ERR3932505	29	19	25	1	28	6	2	118	*C. diphtheriae*
ERR3932506	2	1	33	28	11	2	3	147	*C. diphtheriae*
ERR3932507	19	9	4	2	3	3	2	121	*C. diphtheriae*
ERR3932508	3	4	3	1	4	3	2	11	*C. diphtheriae*
ERR3932510	2	10	9	1	3	27	2	130	*C. diphtheriae*
ERR3932511	19	8	11	9	3	3	5	665	*C. diphtheriae*
ERR3932512	2	4	4	1	3	3	7	9	*C. diphtheriae*
ERR3932515	16	4	8	1	7	16	9	46	*C. diphtheriae*
ERR3932516	7	8	12	10	10	6	4	666	*C. diphtheriae*
ERR3932517	32	22	45	5	18	3	6	133	*C. diphtheriae*
ERR3932518	30	4	28	25	3	3	6	135	*C. diphtheriae*
ERR3932519	3	2	1	1	13	3	2	152	*C. diphtheriae*
ERR3932520	4	11	4	19	3	29	9	134	*C. diphtheriae*
ERR3932521	36	10	3	1	7	3	22	165	*C. diphtheriae*
ERR3932523	8	2	16	1	3	3	12	26	*C. diphtheriae*
ERR3932524	3	4	3	6	3	3	3	141	*C. diphtheriae*
ERR3932525	8	3	19	2	7	3	3	40	*C. diphtheriae*
ERR3932526	4	1	49	5	11	3	6	148	*C. diphtheriae*
ERR3932527	4	2	2	1	2	2	2	155	*C. diphtheriae*
ERR3932528	2	10	24	1	3	31	2	157	*C. diphtheriae*
ERR3932529	4	20	44	33	29	1	21	119	*C. diphtheriae*

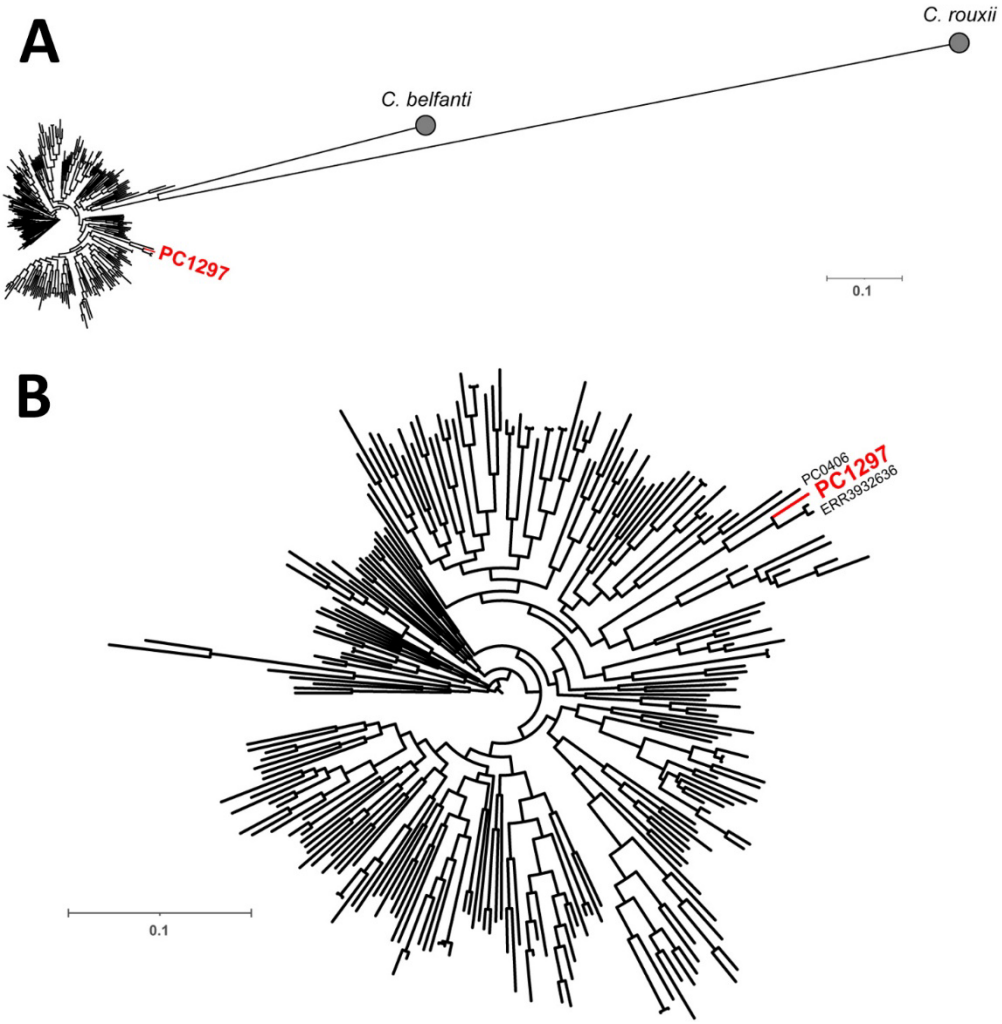
ID	atpA	dnaE	dnaK	fusA	leuA	odhA	rpoB	ST	Species (animal)†
ERR3932530	3	2	13	35	3	2	4	149	*C. diphtheriae*
ERR3932532	14	2	23	4	2	14	2	44	*C. diphtheriae*
ERR3932533	3	2	3	1	33	16	18	154	*C. diphtheriae*
ERR3932534	3	3	3	2	3	3	3	3	*C. diphtheriae*
ERR3932537	3	5	6	5	3	3	6	8	*C. diphtheriae*
ERR3932538	3	4	4	1	3	3	5	117	*C. diphtheriae*
ERR3932539	16	4	8	1	3	30	9	158	*C. diphtheriae*
ERR3932540	4	2	5	2	3	3	5	6	*C. diphtheriae*
ERR3932541	32	1	45	5	11	3	6	145	*C. diphtheriae*
ERR3932542	1	1	1	1	1	78	1	671	*C. diphtheriae*
ERR3932543	9	4	13	11	3	3	9	21	*C. diphtheriae*
ERR3932545	4	21	2	1	3	3	3	127	*C. diphtheriae*
ERR3932546	31	4	48	1	7	1	9	129	*C. diphtheriae*
ERR3932547	2	4	8	1	7	3	9	258	*C. diphtheriae*
ERR3932548	19	9	46	19	3	26	22	126	*C. diphtheriae*
ERR3932549	22	4	138	1	18	3	4	672	*C. diphtheriae*
ERR3932554	30	4	35	25	11	3	6	124	*C. diphtheriae*
ERR3932555	2	10	29	1	12	2	13	143	*C. diphtheriae*
ERR3932557	2	1	39	19	13	24	13	96	*C. diphtheriae*
ERR3932558	2	1	6	29	3	3	2	91	*C. diphtheriae*
ERR3932560	3	1	5	2	3	23	2	88	*C. diphtheriae*
ERR3932564	2	1	38	29	3	3	2	87	*C. diphtheriae*
ERR3932568	19	10	61	4	5	5	2	206	*C. diphtheriae*
ERR3932569	2	4	4	29	3	16	2	205	*C. diphtheriae*
ERR3932570	3	1	13	16	38	2	9	212	*C. diphtheriae*
ERR3932574	2	10	56	45	11	3	3	228	*C. diphtheriae*
ERR3932575	2	1	9	19	3	17	2	667	*C. diphtheriae*
ERR3932576	2	1	9	29	3	3	2	237	*C. diphtheriae*
ERR3932579	3	4	67	29	3	16	2	235	*C. diphtheriae*
ERR3932580	2	9	6	29	3	3	2	238	*C. diphtheriae*
ERR3932581	11	1	20	16	3	3	14	239	*C. diphtheriae*
ERR3932584	2	9	9	29	3	3	2	241	*C. diphtheriae*
ERR3932585	32	1	70	19	3	3	18	250	*C. diphtheriae*
ERR3932587	13	2	44	41	29	1	21	366	*C. diphtheriae*
ERR3932589	2	9	4	59	3	3	2	424	*C. diphtheriae*
ERR3932590	3	2	4	1	3	3	13	136	*C. diphtheriae*
ERR3932594	3	4	11	2	3	3	2	426	*C. diphtheriae*
ERR3932595	22	2	4	29	3	40	2	268	*C. diphtheriae*
ERR3932600	3	2	73	4	43	22	2	274	*C. diphtheriae*
ERR3932601	28	2	38	19	13	3	6	269	*C. diphtheriae*
ERR3932602	2	8	63	30	85	3	19	673	*C. diphtheriae*
ERR3932603	2	10	69	4	5	11	2	668	*C. diphtheriae*
ERR3932604	4	4	96	7	86	57	32	674	*C. diphtheriae*
ERR3932606	11	1	85	2	3	3	2	356	*C. diphtheriae*
ERR3932608	22	9	43	7	3	3	2	109	*C. diphtheriae*
ERR3932611	4	1	20	19	13	16	9	361	*C. diphtheriae*
ERR3932612	25	1	6	19	3	16	6	425	*C. diphtheriae*
ERR3932613	2	23	32	1	55	5	43	362	*C. diphtheriae*
ERR3932614	28	12	6	19	3	51	6	352	*C. diphtheriae*
ERR3932616	2	1	61	19	3	16	2	355	*C. diphtheriae*
ERR3932617	24	15	86	19	11	3	18	374	*C. diphtheriae*
ERR3932618	3	2	35	1	18	3	2	86	*C. diphtheriae*
ERR3932621	46	10	60	7	3	3	2	375	*C. diphtheriae*
ERR3932624	3	8	9	1	3	40	2	310	*C. diphtheriae*
ERR3932625	3	1	12	1	42	16	31	259	*C. diphtheriae*
ERR3932626	2	26	4	4	62	3	2	421	*C. diphtheriae*
ERR3932627	3	8	8	30	3	4	19	95	*C. diphtheriae*
ERR3932630	1	2	9	57	3	3	2	419	*C. diphtheriae*
ERR3932632	3	2	70	1	7	3	2	595	*C. diphtheriae*
ERR3932633	3	3	92	2	3	3	3	387	*C. diphtheriae*
ERR3932634	2	2	38	58	3	3	22	422	*C. diphtheriae*
ERR3932636	18	10	27	21	20	5	16	669	*C. diphtheriae*
ERR3932638	2	2	4	1	3	3	46	388	*C. diphtheriae*
ERR3932639	3	8	9	30	3	4	19	413	*C. diphtheriae*
ERR3932640	2	1	61	4	5	5	2	423	*C. diphtheriae*
ERR3932642	3	1	38	4	3	3	2	410	*C. diphtheriae*
ERR3932643	2	2	2	3	11	64	4	536	*C. diphtheriae*
ERR3932644	4	2	104	30	3	51	2	511	*C. diphtheriae*
ERR3932645	2	26	55	29	30	3	2	414	*C. diphtheriae*

ID	atpA	dnaE	dnaK	fusA	leuA	odhA	rpoB	ST	Species (animal)†
ERR3932647	22	2	4	16	3	40	2	415	*C. diphtheriae*
ERR3932648	25	16	35	44	3	3	28	416	*C. diphtheriae*
ERR3932649	2	2	42	31	11	3	4	100	*C. diphtheriae*
ERR3932651	2	8	32	19	11	17	18	429	*C. diphtheriae*
ERR3932652	19	1	95	2	3	16	6	420	*C. diphtheriae*
ERR3932653	2	8	4	1	3	3	6	430	*C. diphtheriae*
ERR3932655	4	1	2	19	25	16	18	101	*C. diphtheriae*
ERR3932656	4	12	4	1	18	3	13	308	*C. diphtheriae*
ERR3932657	3	9	4	1	3	3	5	417	*C. diphtheriae*
ERR3932658	2	1	60	4	3	3	18	431	*C. diphtheriae*
ERR3932660	30	2	32	19	24	16	9	418	*C. diphtheriae*
ERR3932661	2	1	20	19	3	3	14	295	*C. diphtheriae*
ERR3932662	2	2	92	13	8	3	5	411	*C. diphtheriae*
ERR3932667	19	32	38	82	44	2	20	675	*C. diphtheriae*
ERR3932668	1	2	9	7	73	3	2	549	*C. diphtheriae*
ERR3932673	1	48	139	7	3	57	9	676	*C. diphtheriae*
ERR3932674	13	16	35	44	29	3	28	517	*C. diphtheriae*
ERR3932676	2	1	6	1	3	40	14	481	*C. diphtheriae*
ERR3932678	2	2	3	2	3	3	5	519	*C. diphtheriae*
ERR3932680	3	5	5	2	3	23	20	520	*C. diphtheriae*
ERR3932681	2	1	111	1	5	40	2	532	*C. diphtheriae*
ERR3932682	48	44	88	1	60	1	6	380	*C. diphtheriae*
ERR3932685	33	57	112	44	3	1	13	533	*C. diphtheriae*
ERR3932686	1	1	32	1	70	5	30	522	*C. diphtheriae*
ERR3932687	4	55	5	19	5	59	2	521	*C. diphtheriae*
ERR3932690	2	1	55	25	55	3	2	490	*C. diphtheriae*
ERR3932693	13	29	108	64	60	1	23	524	*C. diphtheriae*
ERR3932697	2	1	47	19	13	3	6	128	*C. diphtheriae*
ERR3932700	2	4	101	7	3	3	32	487	*C. diphtheriae*
ERR3932701	28	9	4	64	3	3	2	526	*C. diphtheriae*
ERR3932702	4	2	109	65	3	51	2	527	*C. diphtheriae*
ERR3932705	5	2	70	19	33	3	53	530	*C. diphtheriae*
ERR3932707	3	1	9	5	3	4	36	320	*C. diphtheriae*
ERR3932708	3	2	73	4	4	22	4	427	*C. diphtheriae*
ERR4332857	3	1	20	7	13	54	2	724	*C. diphtheriae*
ERR4332868	3	1	20	2	13	54	2	384	*C. diphtheriae*
ERR4332886	4	46	4	1	3	3	2	447	*C. diphtheriae*
ERR4332890	2	9	96	2	3	3	4	725	*C. diphtheriae*
ERR4332892	2	12	4	1	3	3	2	292	*C. diphtheriae*
SRR10054224	2	9	1	1	13	3	2	76	*C. diphtheriae*
SRR10054230	3	1	150	19	13	3	48	739	*C. diphtheriae*
SRR11184121	3	1	107	19	13	3	48	652	*C. diphtheriae*
SRR11184124	2	4	6	19	13	3	6	625	*C. diphtheriae*
SRR11184125	2	1	76	19	13	24	13	317	*C. diphtheriae*
SRR11184130	2	1	20	19	42	16	13	244	*C. diphtheriae*
SRR11184131	2	4	137	81	13	3	6	662	*C. diphtheriae*
SRR11184133	3	46	3	6	30	3	2	439	*C. diphtheriae*
SRR11184136	38	1	77	4	5	3	9	319	*C. diphtheriae*
SRR11184138	33	2	4	1	3	3	2	655	*C. diphtheriae*
SRR11184142	3	1	45	1	5	3	9	654	*C. diphtheriae*
SRR11184144	4	1	136	5	11	1	6	661	*C. diphtheriae*
SRR11184153	3	2	131	71	7	2	4	653	*C. diphtheriae*
SRR11184159	2	1	35	29	3	3	2	623	*C. diphtheriae*
SRR11184162	2	1	45	46	5	16	9	246	*C. diphtheriae*
SRR11184166	2	3	92	2	3	3	3	658	*C. diphtheriae*
SRR11184167	3	1	18	4	13	3	5	32	*C. diphtheriae*
SRR11184172	2	3	38	64	7	3	2	657	*C. diphtheriae*
SRR11184173	2	1	35	19	13	24	13	257	*C. diphtheriae*
SRR11184176	1	9	46	19	3	3	22	656	*C. diphtheriae*
SRR12235651 (PC0112)	2	2	4	1	3	3	2	50	*C. diphtheriae*
SRR12235663	3	2	4	15	4	4	4	31	*C. diphtheriae*
SRR12235668 (PC0153)	2	10	24	1	3	3	2	53	*C. diphtheriae*
SRR12235672 (PC0132)	2	10	29	1	12	10	13	38	*C. diphtheriae*
SRR12235677 (PC0104)	10	8	16	14	10	3	9	30	*C. diphtheriae*

ID	atpA	dnaE	dnaK	fusA	leuA	odhA	rpoB	ST	Species (animal)†
SRR12270034 (PC0652)	3	2	60	83	3	3	2	NT	*C. diphtheriae*
SRR12270040 (PC0646)	3	2	60	30	3	3	2	445	*C. diphtheriae*
SRR6816560	59	4	8	53	3	5	13	0	*C. diphtheriae*
SRR6816561	1	16	32	1	87	1	4	687	*C. diphtheriae*
SRR6816562	49	4	8	53	3	5	13	729	*C. diphtheriae*
SRR6816563	32	1	45	45	11	3	3	240	*C. diphtheriae*
SRR6816564	3	4	8	1	18	3	9	498	*C. diphtheriae*
SRR6816565	2	4	4	1	3	3	5	5	*C. diphtheriae*
SRR6816566	1	1	88	44	97	89	13	749	*C. diphtheriae*
SRR6816567	13	29	108	64	60	1	65	744	*C. diphtheriae*
SRR6816568	2	1	33	28	11	2	22	516	*C. diphtheriae*
SRR6816570	4	1	8	1	11	3	6	122	*C. diphtheriae*
SRR6816572	18	1	27	21	20	5	16	59	*C. diphtheriae*
SRR6816573	1	44	9	1	26	69	4	605	*C. diphtheriae*
SRR6816574	13	2	46	19	60	26	13	730	*C. diphtheriae*
SRR6816576	19	1	9	1	18	88	13	745	*C. diphtheriae*
SRR6816578	2	1	35	4	37	3	9	731	*C. diphtheriae*
SRR6816579	25	1	33	28	11	3	22	737	*C. diphtheriae*
SRR6816582	9	44	88	1	60	1	6	734	*C. diphtheriae*
SRR6816583	1	2	66	88	93	5	21	738	*C. diphtheriae*
SRR6816585	2	2	13	1	3	3	13	727	*C. diphtheriae*
SRR6816587	13	2	44	1	95	1	13	747	*C. diphtheriae*
SRR6816590	13	2	32	33	93	1	21	748	*C. diphtheriae*
SRR6816591	49	4	9	53	3	5	13	381	*C. diphtheriae*
SRR6816592	25	16	35	46	2	3	4	732	*C. diphtheriae*
SRR6816596	13	1	20	1	3	5	2	733	*C. diphtheriae*
SRR6816598	3	1	3	1	3	5	13	735	*C. diphtheriae*
SRR6816599	3	1	152	4	13	3	5	750	*C. diphtheriae*
SRR6816601	8	3	3	2	7	3	3	20	*C. diphtheriae*
SRR6816606	1	1	33	1	13	3	6	736	*C. diphtheriae*
SRR7039157	2	12	54	13	3	3	4	297	*C. diphtheriae*
SRR7039160	3	8	94	4	3	4	2	494	*C. diphtheriae*
SRR7039165	19	11	4	19	3	29	9	495	*C. diphtheriae*
SRR7039167	4	2	4	1	3	3	2	446	*C. diphtheriae*
SRR7039176	4	2	4	19	3	16	2	496	*C. diphtheriae*
SRR7039185	3	2	4	1	3	3	2	389	*C. diphtheriae*
SRR7039187	3	1	4	19	13	3	48	441	*C. diphtheriae*
SRR7039194	2	1	20	19	69	3	14	509	*C. diphtheriae*
SRR7039204	2	1	32	19	13	3	50	508	*C. diphtheriae*
SRR7039222	3	1	12	1	68	3	31	507	*C. diphtheriae*
SRR7223801	19	4	8	1	3	3	13	125	*C. diphtheriae*
SRR7825350	22	54	104	16	3	2	2	512	*C. diphtheriae*
SRR7825351	4	4	105	1	3	3	2	513	*C. diphtheriae*
SRR7825371	8	8	3	1	7	3	2	497	*C. diphtheriae*
SRR7825427	3	1	94	1	3	3	2	500	*C. diphtheriae*
SRR8417825	2	12	125	1	3	3	2	586	*C. diphtheriae*
PC1297	18	10	30	21	20	5	16	705	*C. diphtheriae* (cat)
PC0406	3	1	110	19	20	61	16	528	*C. diphtheriae* (dog)
ERR3574992	37	25	53	39	36	35	17	181	*C. rouxii*
ERR3574995	37	25	113	66	72	35	17	538	*C. rouxii*
SRR12235660 (PC0231)	20	14	31	26	22	21	17	74	*C. rouxii* (cat)
SRR12235661 (PC0230)	20	14	31	26	22	21	17	74	*C. rouxii* (cat)
SRR12235662 (PC0229)	20	14	31	26	22	21	17	74	*C. rouxii* (cat)
SRR12235664 (PC0226)	20	14	31	26	22	21	17	74	*C. rouxii* (cat)
PC0751	37	25	91	54	61	21	17	537	*C. rouxii* (dog)
PC1315	37	25	91	26	83	35	3	0	*C. rouxii* (dog)
ERR2094320	6	17	10	12	9	7	11	107	*C. belfantii*
ERR2094321	6	7	10	8	34	13	10	177	*C. belfantii*
ERR2094323	6	7	10	12	9	12	10	106	*C. belfantii*
ERR2094328	6	7	10	18	56	12	11	365	*C. belfantii*
ERR2094329	6	7	10	8	9	13	10	92	*C. belfantii*
ERR2757918	6	7	62	37	9	12	10	208	*C. belfantii*
SRR11184122	6	7	134	63	9	7	10	659	*C. belfantii*

ID	atpA	dnaE	dnaK	fusA	leuA	odhA	rpoB	ST	Species (animal)†
SRR11184123	6	7	10	12	9	7	10	81	*C. belfantii*
SRR11184129	6	7	10	12	84	13	15	663	*C. belfantii*
SRR11184141	6	7	10	18	9	13	10	170	*C. belfantii*
SRR11184154	6	7	135	8	9	77	10	660	*C. belfantii*
SRR11184155	6	7	21	12	9	12	11	409	*C. belfantii*
SRR11184157	6	7	10	37	9	12	11	353	*C. belfantii*
SRR11184171	6	7	10	37	9	12	10	226	*C. belfantii*
SRR7039177	6	7	21	61	9	7	10	501	*C. belfantii*
SRR7039197	6	7	10	8	9	12	11	294	*C. belfantii*

ST, sequence type
†When applicable



Appendix Figure. Phylogenetic reconstruction of *C. diphtheriae* PC1297 and 273 publicly available isolate sequences from 252,766 core SNP sites using maximum likelihood. Cat isolate PC1297 A) was placed within the main clade of typical *C. diphtheriae* strains, distinct from related novel species *C. belfanti* and *C. rouxii* previously associated with domestic dog and cat isolates, and B) was most closely related to human isolate ERR3932636 and shared a branch with dog isolate PC0406. Scale bars indicates substitutions per site.