JCM
Journals.ASM.org

Multilocus Sequence Typing of *Corynebacterium ulcerans* Provides Evidence for Zoonotic Transmission and for Increased Prevalence of Certain Sequence Types among Toxigenic Strains

Christina König,[a] Dominik M. Meinel,[a] Gabriele Margos,[a] Regina Konrad,[a,b] Andreas Sing[a,b]

LGL, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany[a]; National Consiliary Laboratory on Diphtheria, Oberschleissheim, Germany[b]

Human-to-human-transmitted *Corynebacterium diphtheriae* was historically the main pathogen causing diphtheria and has therefore been studied extensively in the past. More recently, diphtheria caused by toxigenic *Corynebacterium ulcerans* is an emerging disease in several industrial countries, including the United Kingdom, the United States, France, and Germany. However, toxigenic *C. ulcerans* has so far been almost neglected in the development of epidemiologic tools. One of the most important tools in modern epidemiology to understand transmission pathways is sequence typing of pathogens. Here, we provide a protocol for multilocus sequence typing (MLST) to type *C. ulcerans* strains rapidly and relatively cost-effectively. Applying MLST to *C. ulcerans* for the first time, we show that related sequence types (STs) might be associated with the presence of the diphtheria toxin gene, which encodes diphtheria toxin (DT), the most important diphtheria-causing virulence factor. Interestingly, we found only two very closely related STs in the isolates derived from six dogs. Additionally, our data show that all STs derived from animals which were at least twice present in our analysis were found in humans as well. This finding is congruent with zoonotic transmission of *C. ulcerans*.

Diphtheria and diphtheria-like diseases are severe infectious diseases which can be caused by three species of the genus *Corynebacterium* (1). Diphtheria is caused by the local and systemic action of diphtheria toxin (DT), which is one of the most potent toxins produced by bacteria (2). DT enters the eukaryotic cell by endocytosis and carries out its catalytic function in the cytoplasm. DT ribosylates the translation factor EF-2 and leads to translational shutdown and thereby cell death (2). The diphtheria disease-causing cluster of *Corynebacterium* is formed by three species: *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* (1). It has been shown for all three species that a nontoxigenic *Corynebacterium* strain can be transformed by integration of a toxigenic phage into the bacterial chromosome (3). In the past, the main pathogen for diphtheria, *C. diphtheriae*, was extensively characterized. *C. diphtheriae* is nearly exclusively transmitted from human to human, making it necessary to develop epidemiologic tools to understand and to combat outbreaks. These efforts resulted in the development of a multilocus sequence typing (MLST) system (4), which enables fast and economical epidemiologic studies and outbreak analysis of *C. diphtheriae* strains. MLST is a very advantageous technique, since it offers high resolution and can be performed very rapidly. MLST is technically relatively undemanding in comparison to other techniques, such as pulsed-field gel electrophoresis or the former gold standard ribotyping. Additionally, MLST is sequence/allele based, and therefore, the resulting data can be shared conveniently with other scientists as the data can be organized, maintained, and searched in public databases, making MLST a perfect tool of choice for fast and accurate typing of transmission pathways.

Although an MLST scheme was published for *C. diphtheriae* (4), no such protocol has been published for *C. ulcerans*, leaving a gap in the epidemiological toolbox. However, in the last several decades *C. ulcerans* was recognized as an emerging pathogen causing diphtheria-like disease. This tendency further increased within the last several years and in many industrialized countries, including the United Kingdom (5), France (6), the United States of America (7), and Germany (8); the infections caused by toxigenic *C. ulcerans* even outnumbered diphtheria cases caused by *C. diphtheriae*. In marked contrast to *C. diphtheriae*, which to date has been almost exclusively isolated from humans, *C. ulcerans* is often found in domestic animals. In addition, *C. ulcerans* has so far not been described to be transmitted from human to human. Therefore, it is thought that the transmission pathways might be different for the two species. Among the animals described to be colonized with *C. ulcerans* are cats, dogs, and pigs (9–13), as well as nondomestic animals, such as cynomolgus macaques (14), ferrets (15), and game animals (16). Although *C. ulcerans* is considered a zoonotic pathogen, molecular indication for zoonotic transmission has been achieved in only four instances, two of them involving dogs (12, 17), one involving a cat (9), and one involving a pig (13).

Considering the fact that toxigenic *C. ulcerans* is gaining greater importance as a diphtheria-causing pathogen, we aimed to establish an MLST scheme for *C. ulcerans* to enhance the epidemiological research and outbreak analysis of *C. ulcerans*.

Received 11 August 2014 **Returned for modification** 20 September 2014
Accepted 2 October 2014
Published ahead of print 15 October 2014
Editor: D. J. Diekema
Address correspondence to Andreas Sing, andreas.sing@lgl.bayern.de.
C.K. and D.M.M. contributed equally to this work.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.02291-14.

doi:10.1128/JCM.02291-14

TABLE 1 Oligonucleotide sequences of the primers used for amplification and sequencing for MLST of *C. ulcerans* with the calculated amplicon size

Primer name	Sequence (5′–3′)	Amplicon/MLST fragment size (bp)	Reference
atpA_fwd	GCGATTGCGAACTACACC	1,029/378	4
atpA_rev	CTCGAGGAATACCTRACC		4
dnaE_fwd	TGATTATGGCCAGCGTKC	581/354	4
dnaE_rev	ACCCATGGCYTTACGGAA		4
fusA_fwd	TACCGCGAGAAGCTCGTT	683/360	4
fusA_rev	GAAGGTTGGGTCCTCTTC		4
odhA_fwd	CGGCAAGGAAASCATGAC	505/381	4
odha_rev	GTTGTCGCCTAACATCTG		4
rpoB_fwd	AAGCGCAAGATCCAGGAC	845/342	4
rpoB_rev	TCGAACTCGTCGTCATCC		4
Culc_dnaK_fwd	ACTTGGGTGGCGGAACCT	687/345	This study
Culc_dnaK_rev	TGGTAAAGGTCTCAGAA		This study
Culc_leuA_fwd	CGTTCACTTCTACAATTC	864/384	This study
Culc_leuA_rev	GCCGTGGTCAGTTTTCAT		This study

MATERIALS AND METHODS

Bacterial strain collection. Forty-four isolates originating from human patients ($n = 31$) or from animals ($n = 13$) from the *C. ulcerans* collection of the National Consiliary Laboratory on diphtheria (NCLoD), as well as published whole-genome sequences, were analyzed. The strains were derived from patient isolates which were sent to the NCLoD from clinical microbiology laboratories for further differentiation and testing. Species were determined using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry and/or biochemical testing (API Corynebacterium). Additionally, the *rpoB* gene was partially sequenced and the isolates were tested for toxigenicity by *tox*-PCR as described previously (18).

DNA preparation. *C. ulcerans* isolates were grown on plates overnight at 37°C. One colony was picked, and DNA was prepared using a Biosprint device (Qiagen) according to the manufacturer's instructions. DNA was quantified using a NanoDrop photometer (Thermo Scientific).

Locus amplification and sequencing. Each PCR was carried out in a 50-μl total volume using HotStarTaq Master Mix (Qiagen). Sequences of the primers and the expected amplicon sizes are given in Table 1. Primers for *atpA*, *dnaA*, *fusA*, *odhA*, and *rpoB* are identical with ones from reference 4. The primer used for *dnaK* and *leuA* was adapted to *C. ulcerans* according to the genome of *C. ulcerans* 809 (19). Locus amplification for MLST analysis was performed according to the published scheme for *C. diphtheriae* with minor modifications (4). DNA was amplified in a thermal cycler (Eppendorf) with the following conditions for *atpK*, *dnaE*, *dnaK*, *fusA*, *odhA*, and *rpoB*: 95°C for 15 min and 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min followed by 72°C for 5 min. *leuA* was amplified using a touchdown PCR: after 95°C for 15 min, 10 cycles of 94°C for 1 min, 60°C to 50°C (minus 1°C per cycle) for 1 min, and 72°C for 2 min. These 10 cycles were followed by 25 cycles with 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min with a final extension at 72°C for 5 min.

The size of amplicons was estimated by agarose gel electrophoresis. The PCR products were sent to Source BioScience (Berlin, Germany) for purification and sequencing. Each product was sequenced using the forward and reverse primers, which was also used for the PCR amplification.

Allele numbers were assigned to each unique allele for a given locus. For each isolate, the allelic profile was generated by combining the allele numbers for each locus in the order *atpA*, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA*, and *rpoB*. The sequenced loci are homologous to the loci used for *C. diphtheriae* (4), giving the possibility of comparing the alleles between the two species.

MLST analysis. Allele and sequence type number were assigned and used for goeBURST analysis using PHYLOViZ (20). For phylogenetic analysis, the MLST sequences were concatenated and aligned using Clustal W2 (21). Neighbor-joining (NJ) trees and bootstrapping were calculated using MEGA 6.0 (22).

RESULTS

Allelic variation. In order to establish an MLST procedure for *C. ulcerans* which is applicable for epidemiological research, outbreak analysis, and identification of ST, we chose the orthologous genes used previously for the *C. diphtheriae* MLST (4), to ensure—if needed—cross-species comparability. The primers used for *atpA*, *dnaA*, *fusA*, *odhA*, and *rpoB* are identical with the ones used for *C. diphtheriae*. The primers used for *dnaK* and *leuA* were adapted to *C. ulcerans* according to the genome of *C. ulcerans* 809 (19) and are given in Table 1. We were able to amplify all MLST loci, *atpA* (1,029 bp), *dnaA* (581 bp), *fusA* (683 bp), *odhA* (505 bp), *rpoB* (845 bp), *dnaK* (687 bp), and *leuA* (864 bp), using two different PCR cycling conditions. The MLST sequences obtained for all isolates and isolate information can be found in Table S1 in the supplemental material and are deposited at http://pubmlst.org/cdiphtheriae (23).

All alleles obtained had similar sequence lengths, and the minimal identity of the different alleles within *C. ulcerans* varied within the data set presented here from 95% to 99%: *atpA*, 99%; *dnaE*, 96%; *dnaK*, 98%; *fusA*, 97%; *leuA*, 97%; *odhA*, 95%; and *rpoB*, 98%. The identity values obtained for the different loci in *C. ulcerans* in the presented study are very similar to the ones obtained for *C. diphtheriae* (90 to 95%) in the initial published study by Bolt et al. (4). In total, 12 STs were assigned to 33 isolates. We obtained 4 *atpA*, 6 *dnaE*, 5 *dnaK*, 3 *fusA*, 7 *leuA*, 4 *odhA*, and 4 *rpoB* alleles. Additionally, we extracted the sequences of the MLST loci from the published *C. ulcerans* genomic sequences of isolates 809 (NC_017317.1) (19), BR-AD22 (NC_015683) (19), 0102 (NC_018101.1) (24), and FRC58 (NZ_AYTI00000000) (25) as well as from 7 isolates sequenced in one of the projects at the NCLoD (D. M. Meinel, G. Margos, R. Konrad, S. Krebs, H. Blum, and A. Sing, unpublished data). Allele comparison led to the identification of one additional allele for *dnaE* and *leuA* as well as two for *odhA*, resulting in three new STs.

We assessed whether *C. ulcerans* and *C. diphtheriae* share common alleles due to their close relationship. As expected, the loci are very similar between the two species; nevertheless, we did not detect any shared allele within the first presented MLST data set for *C. ulcerans*. We detected identities of approximately 86 to 89% for *atpA*, *dnaK*, *fusA*, and *rpoB*. For *odhA*, we detected 80 to 82% identity, and for the *leuA* alleles, we detected a minimum of 78%, illustrating the separation of the two species and the idea that the

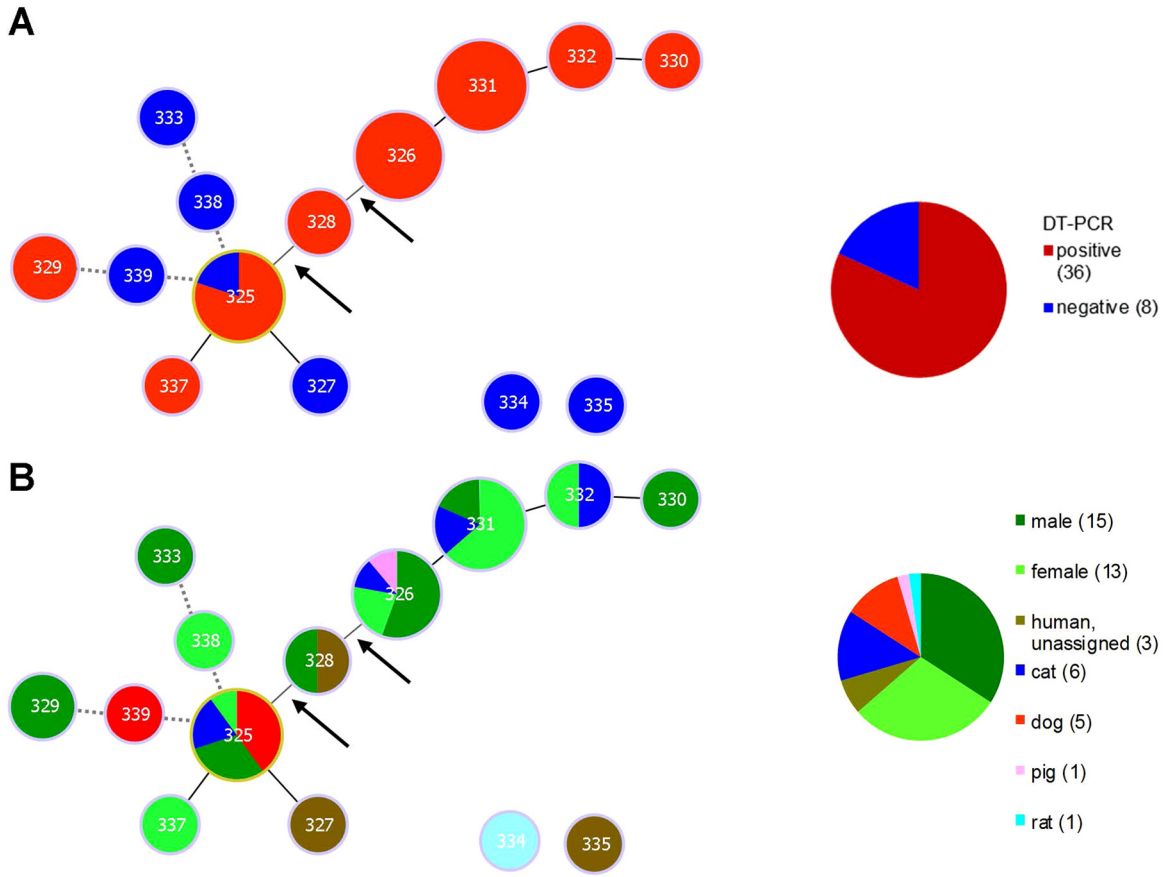

FIG 1 goeBURST diagram for the MLST data set of 44 *C. ulcerans* isolates. (A) An eBURST diagram was calculated using PHYLOViZ with the goeBURST algorithm. STs were grouped according to their allelic profiles. Solid lines indicate single-locus variants, except for the two lines marked with arrows, which indicate double-locus variants. The dotted lines reflect triple-locus variants and therefore more distantly related isolates. Each circle represents one ST, and the size represents the number of isolates in each ST. Exact numbers of isolates per ST are given in Table 2. Isolates which tested positive for the *tox* gene by PCR are colored red; negative isolates are colored blue. In the right panel, a pie chart depicts the fractions of *tox*-positive and -negative strains in the analysis. (B) eBURST diagram tree as in panel A. The isolates are color coded according to their host, as given in the key at right. The pie chart depicts the isolates from each host as fractions of the total number of isolates.

species can be easily discriminated from each other by the alleles obtained with MLST.

goeBurst analysis. For a more detailed analysis of the population structure, we used PHYLOViZ to generate goeBurst diagrams of the typed isolates. First, we analyzed whether certain STs carry the DT gene more often. Interestingly, approximately 81% of the isolates analyzed carried a *tox* gene. We found very distinct STs in the different non-*tox*-bearing isolates (Fig. 1A). In contrast, the toxigenic isolates seem to form a lineage which carries the *tox* gene more often, suggesting that there might be an ST which is more susceptible to horizontal gene transfer by toxigenic transformation or that the DT genes are passed on over several generations in that lineage. Notably, this effect is more visible if not only single-locus variants are allowed in each lineage but also double-locus variants are considered: ST 325 and 326 get connected remotely via ST 328 (connections of double-locus variants are marked with an arrow in Fig. 1A). If triple-locus variants are allowed, STs 325, 339, and 329 and STs 325, 338, and 333 will be connected (dotted lines in Fig. 1A). STs 334 and 335 appeared as singletons showing no connection to other STs.

In the next step, we analyzed whether certain ST lineages are species specific. Since *C. ulcerans* is suspected to be transmitted zoonotically, we assessed whether all STs are present in both animals and humans (Fig. 1B). We found that, indeed for all STs for which more than two isolates were available, at least one human isolate was present, supporting the concept of zoonotic transmission for *C. ulcerans*. Furthermore, we found several STs in the isolates originating from cats, indicating no visible ST specificity. Interestingly, we found 4 of 5 isolates originating from dogs to have the same ST. The remaining fifth isolate (ST 339) showed two allelic changes compared to the 4 isolates with ST 325. ST 339 MLST sequences were extracted from a published whole-genome sequence of an isolate which originated from Brazil and might therefore be expected to be more different from the other isolates, derived from Germany.

Phylogenetic analysis. We also used the obtained sequence data to generate a phylogenetic tree. Concatenated sequences of all seven loci were used to calculate a neighbor-joining (NJ) tree. As already obvious from the goeBURST analysis, KL349 and KL274, which correspond to STs 335 and 334, respectively, are most distant from the other isolates (Fig. 2). The other isolates are very similar to each other (see Table S1 in the supplemental material), and we did not observe any strong lineages. As expected, all STs form exclusive clusters in the phylogenetic trees.

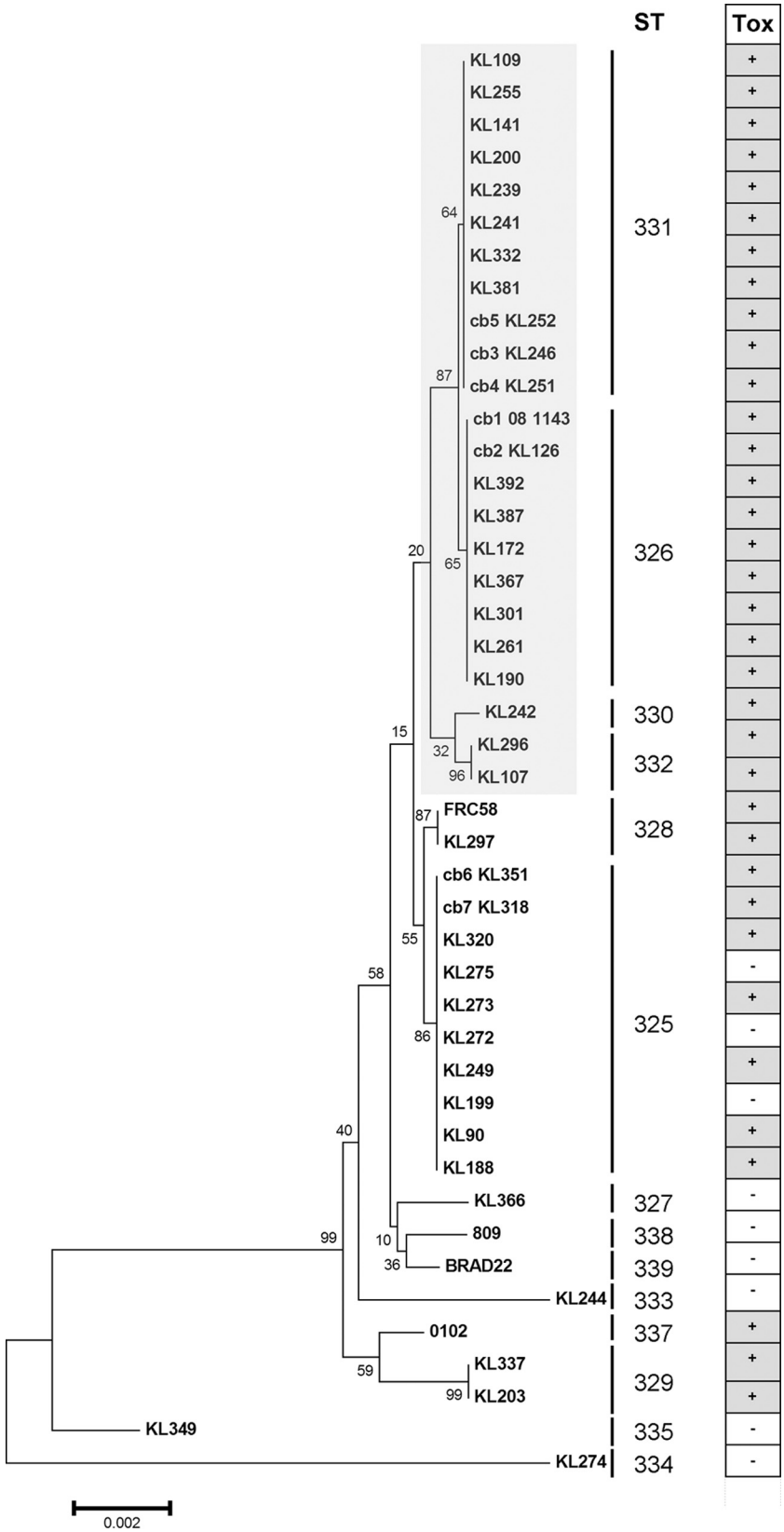

FIG 2 Neighbor-joining tree based on concatenated MLST sequencing data for 44 *C. ulcerans* isolates. The NJ tree was calculated using MEGA 6.0 (22). The strain identifiers are given at the ends of the branches. The numbers give the values for the bootstrapping test of the tree with 100 repetitions. The sequence type is given under "ST," and the results of the *tox*-PCR are given in the rightmost panel. The lineage of STs 326, 330, 331, and 332 is highlighted in light gray. All isolates in this cluster were *tox* positive.

TABLE 2 *C. ulcerans* isolates analyzed in this study

ST	Isolate	Yr of isolation	Place of isolation	Patient gender or animal	Age of patient (yr)	Result of Elek test: *tox*	*tox*-PCR result	Symptoms/site of colonization	Note
326	KL392	2012	Baden-Württemberg, Germany	Cat	NA[c]	Negative	Positive	Animal, no symptoms detected	From same household as KL387
326	KL387	2012	Baden-Württemberg, Germany	Male	86	Negative	Positive	Wound	From same household as KL392
326	KL172	2009	Baden-Württemberg, Germany	Male	56	Negative	Positive	Wound	
367	KL367	2012	Baden-Württemberg, Germany	Male	80	Negative	Positive	Wound	
326	KL301	2011	Bavaria, Germany	Male	57	Positive	Positive	Wound	
326	KL261	2011	Bavaria, Germany	Male	57	Positive	Positive	Wound/ulcer	
326	KL190	2010	Baden-Württemberg, Germany	Female	61	Positive	Positive	Wound	
327	KL366	2012	Baden-Württemberg, Germany	Human	NA	NA	Negative	NA	
325	KL320	2012	Hesse, Germany	Dog	NA	Negative	Positive	Animal, no symptoms detected	
325	KL275	2011	Baden-Württemberg, Germany	Dog	NA	NA	Negative	Animal	
325	KL273	2011	Baden-Württemberg, Germany	Cat	NA	Positive	Positive	Cat with symptoms	
325	KL272	2011	Baden-Württemberg, Germany	Cat	NA	Negative	Positive	Cat with symptoms	
325	KL249	2010	Berlin, Germany	Dog	NA	Negative	Positive	Animal, no symptoms detected	
325	KL199	2010	North Rhine-Westphalia, Germany	Male	41	NA	Negative	Ethmoid bone	
325	KL90	2005	Bavaria, Germany	Female	35	NA	Positive	Throat	
325	KL188	2010	Bavaria, Germany	Male	64	Negative	Positive	Ulcer	
328	KL297	2011	Baden-Württemberg, Germany	Male	74	Negative	Positive	Ulcer	
329	KL337	2012	Bavaria, Germany	Male	58	Positive	Positive	NA	
329	KL203	2010	Baden-Württemberg, Germany	Male	58	Positive	Positive	Wound	
330	KL242	2010	Saxony, Germany	Male	62	Positive	Positive	Ulcer	
331	KL381	2012	Baden-Württemberg, Germany	Male	73	Negative	Positive	Wound	
331	KL332	2012	Bavaria, Germany	Male	72	Negative	Positive	Wound	
331	KL241	2010	Saxony, Germany	Female	63	Positive	Positive	Ulcer	
331	KL239	2010	NA	Female	89	Negative	Positive	Wound	
331	KL200	2010	NA	Female	87	Positive	Positive	Wound	
331	KL141	2009	NA	Female	62	Positive	Positive	Throat	
331	KL109	2007	Thuringia, Germany	Female	NA	Positive	Positive	NA	
331	KL255	2011	Saxony, Germany	Female	85	Negative	Positive	Ulcer	
332	KL296	2011	Hamburg, Germany	Female	66	Negative	Positive	Wound	
332	KL107	2007	Brandenburg, Germany	Cat	NA	Positive	Positive	Animal, no symptoms detected	
333	KL244	2010	NA	Male	58	NA	Negative	Ulcer	
334	KL274	2011	Baden-Württemberg, Germany	Rat	NA	NA	Negative	Animal with symptoms	
335	KL349	2012	London, UK	Human	NA	Negative	Negative	Throat	
337	0102[a]	NA	Japan	Female	52	Positive	Positive	Throat	Reference 24
338	809[a]	NA	Brazil	Female	80	NA	Negative	Lung	Reference 26
339	BR-AD22[a]	NA	Brazil	Dog	5	NA	Negative	Animal with symptoms	Reference 26
326	081143[a]	2007	NA	Pig	NA	NA	Positive	Animal, no symptoms detected	From same household as KL126
326	KL126[a]	2007	NA	Female	56	Positive	Positive	Throat	From same household as 081143

(Continued on following page)

TABLE 2 (Continued)

ST	Isolate	Yr of isolation	Place of isolation	Patient gender or animal	Age of patient (yr)	Result of Elek test: *tox*	*tox*-PCR result	Symptoms/site of colonization	Note
331	KL246[a]	2010	Saxony, Germany	Female	86	Weakly positive	Positive	Throat	From same household as KL251 and KL252
331	KL251[a]	2010	Saxony, Germany	Cat	NA	Negative	Positive	Animal, no symptoms detected	From same household as KL246 and KL252
331	KL252[a]	2010	Saxony, Germany	Cat	NA	Negative	Positive	Animal, no symptoms detected	From same household as KL246 and KL251
325	KL315[a]	2012	Baden-Württemberg, Germany	Male	52	Negative	Positive	Ulcer	From same household as KL318
325	KL318[a]	2012	Baden-Württemberg, Germany	Dog	NA	Negative	Positive	Animal, no symptoms detected	From same household as KL315
328	FRC58[a]	NA	France	Human	86	NA	Positive[b]	Lung	Reference 25

[a] The corresponding MLST sequences were extracted from NGS data.
[b] It is unknown whether a *tox*-PCR was performed, but inspection of NGS resequencing data showed that a full *tox* gene was present.
[c] NA, data not available.

Interestingly, one of the two lineages seen in the goeBURST analysis, consisting of STs 326, 331, 332, and 330, forms a highly homogenous branch in the phylogenetic analysis. Notably, all isolates in this cluster bear a *tox* gene. Furthermore, both isolates of ST 328, which is located close to this cluster in the goeBURST analysis and which represents a double-locus variant of the closest member of the cluster (Fig. 1), bear a *tox* gene. ST 325, a triple-locus variant of the closest member of the cluster, also has a high proportion of toxigenic isolates.

As already noted before upon inclusion of all available MLST sequence data published on PubMLST.org, *C. diphtheriae* and *C. ulcerans* form two highly separated branches in the phylogeny, because although the sequences for the single MLST loci are very similar in the two species, the sequences are still clearly distinguishable from each other. This is expected from two distinct species with low horizontal gene transfer affecting the analyzed loci (data not shown).

DISCUSSION

C. ulcerans is an emerging pathogen in regions which are highly vaccinated against diphtheria. Since human-to-human transmission has so far been reported only anecdotally, most countries have not included toxigenic *C. ulcerans* in their list of notifiable diseases. However, alarming reports on rising numbers of cases of diphtheria and diphtheria-like disease caused by toxigenic *C. ulcerans* from several highly industrialized countries, such as the United Kingdom, the United States, France, and Germany, indicate that the diphtheria cases caused by *C. ulcerans* outnumber those caused by *C. diphtheriae*. Therefore, it might be reasonable to keep toxigenic *C. ulcerans* under closer epidemiological surveillance to gain deeper insight into the public health relevance of this pathogen. To better understand transmission pathways and to learn whether special sequence types of *C. ulcerans* have a higher pathogenic potential, we established MLST for *C. ulcerans* as an epidemiological tool. MLST is a fast and relatively cost-effective technique. The sequence-based MLST data can be stored in public databases, are portable, and can be easily searched and shared between laboratories. Here, we present MLST data for 33 isolates obtained by classical PCR and sequencing and data for 11 isolates extracted from next-generation sequencing (NGS) data, also illustrating the compatibility of MLST with the newly emerging NGS technology.

Our data nicely illustrate the importance of MLST for *C. ulcerans*, by showing that there might be—with the caveat that we analyzed only 44 isolates— certain *C. ulcerans* STs which carry the *tox* gene more often. Whether this is due to a higher susceptibility to horizontal gene transfer for uptake of the *tox* gene in those ST clusters or whether it is due to the *tox* gene being passed on from a common ancestor needs to be further investigated. However, this points to the possibility that certain STs might be connected with higher virulence and with a more severe course of disease. Since *C. ulcerans* can cause diverse clinical pictures, such as skin ulcers and cutaneous and pharyngeal diphtheria, it is of great importance to investigate whether certain STs are associated with particular courses of disease. However, we did not detect a clear connection of STs with the severity of the course of disease. Most likely, a more comprehensive data set is needed for such conclusions, for which the foundations could be easily laid when more and more labs apply MLST to *C. ulcerans* and make their data available.

Additionally, we found that all STs which were present at least twice in our data set and originated from animals were also among the isolates from human patients. This provides an additional indication for zoonotic transmission of *C. ulcerans*. Hence, it underlines the importance of molecular typing of these pathogens to understand transmission pathways between humans and animals as well as human-to-human or animal-to-animal transmission. Our study also included a porcine strain (KL126) which shares the same ST as an isolate obtained from the farmer who owned the pig (cb1 08-1143), as well as isolates from a dog (KL315) and its owner (KL318) both with the same ST, providing molecular evidence for zoonotic transmission (Table 2). Since all four isolates are toxigenic, both cases of likely zoonotic transmission underline that the companion animals of a patient also should be probed and, when needed, treated to avoid possible infection cycles with toxigenic *C. ulcerans*.

ACKNOWLEDGMENTS

We thank Wolfgang Schmidt for the cultivation and microbiological characterization of the corynebacteria and Cecilia Hizo-Teufel for help with the DNA preparation. We thank Edgar Badell-Ocando and Keith Jolley for curation of the http://pubmlst.org database and integration of our MLST data into the *Corynebacterium diphtheriae* database.

The study was partly supported by the Bavarian State Ministry of Health and Care and by the German Federal Ministry of Health via the Robert Koch Institute and its National Reference Laboratories Network (FKZ 1369–359 and FKZ 415).

The authors have declared that no competing interests exist.

REFERENCES

1. **Pascual C, Lawson PA, Farrow JA, Gimenez MN, Collins MD.** 1995. Phylogenetic analysis of the genus Corynebacterium based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. **45:**724–728. http://dx.doi.org/10.1099/00207713-45-4-724.
2. **Holmes RK.** 2000. Biology and molecular epidemiology of diphtheria toxin and the tox gene. J. Infect. Dis. **181:**S156–S167. http://dx.doi.org/10.1086/315554.
3. **Brussow H, Canchaya C, Hardt WD.** 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Rev. **68:**560–602. http://dx.doi.org/10.1128/MMBR.68.3.560-602.2004.
4. **Bolt F, Cassiday P, Tondella ML, Dezoysa A, Efstratiou A, Sing A, Zasada A, Bernard K, Guiso N, Badell E, Rosso ML, Baldwin A, Dowson C.** 2010. Multilocus sequence typing identifies evidence for recombination and two distinct lineages of Corynebacterium diphtheriae. J. Clin. Microbiol. **48:**4177–4185. http://dx.doi.org/10.1128/JCM.00274-10.
5. **Wagner KS, White JM, Crowcroft NS, De Martin S, Mann G, Efstratiou A.** 2010. Diphtheria in the United Kingdom, 1986–2008: the increasing role of Corynebacterium ulcerans. Epidemiol. Infect. **138:**1519–1530. http://dx.doi.org/10.1017/S0950268810001895.
6. **Bonmarin I, Guiso N, Le Fleche-Mateos A, Patey O, Patrick AD, Levy-Bruhl D.** 2009. Diphtheria: a zoonotic disease in France? Vaccine **27:**4196–4200. http://dx.doi.org/10.1016/j.vaccine.2009.04.048.
7. **Tiwari TS, Golaz A, Yu DT, Ehresmann KR, Jones TF, Hill HE, Cassiday PK, Pawloski LC, Moran JS, Popovic T, Wharton M.** 2008. Investigations of 2 cases of diphtheria-like illness due to toxigenic Corynebacterium ulcerans. Clin. Infect. Dis. **46:**395–401. http://dx.doi.org/10.1086/525262.
8. **Sing A.** 2008. Zur Charakterisierung von C.-diphtheriae-verdächtigen Isolaten. Robert Koch Inst. Epidemiol. Bull. **3:**23–25.
9. **Berger A, Huber I, Merbecks SS, Ehrhard I, Konrad R, Hormansdorfer S, Hogardt M, Sing A.** 2011. Toxigenic Corynebacterium ulcerans in woman and cat. Emerg. Infect. Dis. **17:**1767–1769. http://dx.doi.org/10.3201/eid1709.110391.
10. **Corti MA, Bloemberg GV, Borelli S, Kutzner H, Eich G, Hoelzle L, Lautenschlager S.** 2012. Rare human skin infection with Corynebacterium ulcerans: transmission by a domestic cat. Infection **40:**575–578. http://dx.doi.org/10.1007/s15010-012-0254-5.
11. **Katsukawa C, Kawahara R, Inoue K, Ishii A, Yamagishi H, Kida K, Nishino S, Nagahama S, Komiya T, Iwaki M, Takahashi M.** 2009. Toxigenic Corynebacterium ulcerans isolated from the domestic dog for the first time in Japan. Jpn. J. Infect. Dis. **62:**171–172.
12. **Hogg RA, Wessels J, Hart J, Efstratiou A, De Zoysa A, Mann G, Allen T, Pritchard GC.** 2009. Possible zoonotic transmission of toxigenic Corynebacterium ulcerans from companion animals in a human case of fatal diphtheria. Vet. Rec. **165:**691–692.
13. **Schuhegger R, Schoerner C, Dlugaiczyk J, Lichtenfeld I, Trouillier A, Zeller-Peronnet V, Busch U, Berger A, Kugler R, Hormansdorfer S, Sing A.** 2009. Pigs as source for toxigenic Corynebacterium ulcerans. Emerg. Infect. Dis. **15:**1314–1315. http://dx.doi.org/10.3201/eid1508.081568.
14. **Hirai-Yuki A, Komiya T, Suzaki Y, Ami Y, Katsukawa C, Takahashi M, Yamamoto A, Yamada YK.** 2013. Isolation and characterization of toxigenic Corynebacterium ulcerans from 2 closed colonies of cynomolgus macaques (Macaca fascicularis) in Japan. Comp. Med. **63:**272–278.
15. **Marini RP, Cassiday PK, Venezia J, Shen Z, Buckley EM, Peters Y, Taylor N, Dewhirst FE, Tondella ML, Fox JG.** 2014. Corynebacterium ulcerans in ferrets. Emerg. Infect. Dis **20:**159–161. http://dx.doi.org/10.3201/eid2001.130675.
16. **Eisenberg T, Kutzer P, Peters M, Sing A, Contzen M, Rau J.** 2014. Nontoxigenic tox-bearing Corynebacterium ulcerans infection among game animals, Germany. Emerg. Infect. Dis. **20:**448–452. http://dx.doi.org/10.3201/eid2003.130423.
17. **Lartigue MF, Monnet X, Le Fleche A, Grimont PA, Benet JJ, Durrbach A, Fabre M, Nordmann P.** 2005. Corynebacterium ulcerans in an immunocompromised patient with diphtheria and her dog. J. Clin. Microbiol. **43:**999–1001. http://dx.doi.org/10.1128/JCM.43.2.999-1001.2005.
18. **Schuhegger R, Lindermayer M, Kugler R, Heesemann J, Busch U, Sing A.** 2008. Detection of toxigenic Corynebacterium diphtheriae and Corynebacterium ulcerans strains by a novel real-time PCR. J. Clin. Microbiol. **46:**2822–2823. http://dx.doi.org/10.1128/JCM.01010-08.
19. **Trost E, Al-Dilaimi A, Papavasiliou P, Schneider J, Viehoever P, Burkovski A, Soares SC, Almeida SS, Dorella FA, Miyoshi A, Azevedo V, Schneider MP, Silva A, Santos CS, Santos LS, Sabbadini P, Dias AA, Hirata R, Jr, Mattos-Guaraldi AL, Tauch A.** 2011. Comparative analysis of two complete Corynebacterium ulcerans genomes and detection of candidate virulence factors. BMC Genomics **12:**383. http://dx.doi.org/10.1186/1471-2164-12-383.
20. **Francisco AP, Vaz C, Monteiro PT, Melo-Cristino J, Ramirez M, Carrico JA.** 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. BMC Bioinformatics **13:**87. http://dx.doi.org/10.1186/1471-2105-13-87.
21. **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** 2007. Clustal W and Clustal X version 2.0. Bioinformatics **23:**2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404.
22. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30:**2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
23. **Jolley KA, Chan MS, Maiden MC.** 2004. mlstdbNet—distributed multilocus sequence typing (MLST) databases. BMC Bioinformatics **5:**86. http://dx.doi.org/10.1186/1471-2105-5-86.
24. **Sekizuka T, Yamamoto A, Komiya T, Kenri T, Takeuchi F, Shibayama K, Takahashi M, Kuroda M, Iwaki M.** 2012. Corynebacterium ulcerans 0102 carries the gene encoding diphtheria toxin on a prophage different from the C. diphtheriae NCTC 13129 prophage. BMC Microbiol. **12:**72. http://dx.doi.org/10.1186/1471-2180-12-72.
25. **Silva Ado S, Barauna RA, de Sa PC, das Gracas DA, Carneiro AR, Thouvenin M, Azevedo V, Badell E, Guiso N, da Silva AL, Ramos RT.** 2014. Draft genome sequence of Corynebacterium ulcerans FRC58, isolated from the bronchitic aspiration of a patient in France. Genome Announc. **2:**01132–13. http://dx.doi.org/10.1128/genomeA.01132-13.
26. **Trost E, Gotker S, Schneider J, Schneiker-Bekel S, Szczepanowski R, Tilker A, Viehoever P, Arnold W, Bekel T, Blom J, Gartemann KH, Linke B, Goesmann A, Puhler A, Shukla SK, Tauch A.** 2010. Complete genome sequence and lifestyle of black-pigmented Corynebacterium aurimucosum ATCC 700975 (formerly C. nigricans CN-1) isolated from a vaginal swab of a woman with spontaneous abortion. BMC Genomics **11:**91. http://dx.doi.org/10.1186/1471-2164-11-91.