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## Diphtheria in Belgium: 2010–2017

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### Abstract

In Western Europe, the incidence of both respiratory and cutaneous diphtheria, caused by toxin-producing *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* or *Corynebacterium pseudotuberculosis*, has been low over the past few decades thanks to the use of an effective vaccine and a high level of vaccination coverage. However, the disease has still not been eradicated and continues to occur in all of Europe. In order to prevent sequelae or a fatal outcome, diphtheria antitoxin (DAT) should be administered to suspected diphtheria patients as soon as possible, but economic factors and issues concerning regulations have led to poor availability of DAT in many countries. The European Centre for Disease Prevention and Control and World Health Organization have called for European Union-wide solutions to this DAT-shortage. In order to illustrate the importance of these efforts and underline the need for continued diphtheria surveillance, we present data on all registered cases of toxigenic and non-toxigenic *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* in Belgium during the past decade, up to and including 2017.

### INTRODUCTION

The European Union (EU) case definition of diphtheria comprises respiratory and cutaneous diphtheria as well as diphtheria of the conjunctiva and mucous membranes, caused by toxin-producing *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* or *Corynebacterium pseudotuberculosis* [1]. *C. diphtheriae* may be further classified into three different biovars: gravis, mitis and intermedius. This species is the most contagious and spreads easily from person to person, while *C. ulcerans* and *C. pseudotuberculosis* are mostly associated with animal transmission [2, 3]. Previously, a fourth biovar; belfanti, was included, but this biovar was recently classified as a new species *Corynebacterium belfantii* [4].

Typical of classic respiratory diphtheria is the formation of a dense, grey fibrinous layer composed of a mixture of dead cells, fibrin, red and white blood cells, and micro-organisms, on the mucosal membranes of the throat. This pseudomembrane,

combined with swelling of the lymph nodes, may impede airflow and cause respiratory complications. Furthermore, the diphtheria toxin excreted by the colonizing corynebacteria may enter the circulation and cause cardiac and neurological complications. Cutaneous diphtheria, on the other hand, presents as an ulcerative skin lesion and does not typically induce systemic disease [2].

To prevent sequelae or fatal outcome, prompt administration of diphtheria antitoxin (DAT) is critical. In Belgium, administration is officially recommended within 48 h of clinical suspicion of respiratory diphtheria [5]. Diphtheria antitoxin is a polyclonal immunoglobulin preparation derived from equine antiserum and listed among the World Health Organization (WHO) Essential Medicines. However, economic factors as well as issues concerning regulations have led to poor availability in many countries [6–9]. Following a fatal case of diphtheria in Belgium in 2016, the European Centre for Disease Prevention and Control (ECDC) called

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**Keywords:** diphtheria; corynebacteria; *Corynebacterium diphtheriae*; *Corynebacterium ulcerans*; DAT; croup.

**Abbreviations:** API, analytical profile index; CLSI, Clinical and Laboratory Standards Institute; DAT, diphtheria antitoxin; ECDC, European Centre for Disease Prevention and Control; EU, European Union; EUCAST, European Committee on Antimicrobial Susceptibility Testing; IU, international units; MALDI-TOF MS, matrix-assisted laser desorption-ionization time of flight mass spectrometry; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; NRC, National Reference Centre; NTTB, non-toxigenic *tox*-bearing; PCR, polymerase chain reaction; PHE, Public Health England; ST, sequence type; *tox*, diphtheria toxin gene; WHO, World Health Organization.

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for EU-wide solutions to the acute DAT-shortage [10], and DAT is currently available in Belgium through the Belgian Poison Centre [5].

Through the use of an effective vaccine and a high level of vaccination coverage, the incidence of diphtheria in Europe has been low over the last decades. Although the diphtheria vaccine is a toxoid, not expected to prevent colonization, a dramatic decrease in incidence and colonization has been observed in immunized populations. Several causing factors have been hypothesized, such as a natural cyclical occurrence of the disease with long gaps, a lack of selective advantage of toxin production in immunized populations, and other unmeasured immune mechanisms [11].

Despite all this, since the end of the 1980s, numerous epidemics have occurred mainly in Eastern Europe and more recently in numerous other WHO global regions [12, 13]. From 1980 until 1989, 1815 diphtheria cases were registered in the European Region. From 1990 until 1999, this number was as high as 160 384, of which more than 70 % were in the Russian Federation [14]. Sporadic cases continue to occur in the rest of Europe, with 313 registered cases from 2010 until 2017 [2, 14–16]. In Belgium, vaccination coverage for four doses of the diphtheria-tetanus-pertussis vaccine was 93 % in 2016, and for three doses was as high as 98 % [17], whereas adult booster vaccination coverage was estimated at 61 % in 2008 [18]. More recent data on adult vaccination coverage in Belgium are not available, but a booster dose every 10 years is officially recommended [19]. However, according to seroprevalence studies in 2011 and 2016, the diphtheria seroprotection rate is low and decreases with age, with only 55.2 % of the population having an antibody concentration above the protective level of 0.1 IU ml<sup>-1</sup> [20, 21].

The Belgian National Reference Centre (NRC) for toxigenic corynebacteria performs surveillance and typing of all toxigenic *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* occurring in Belgium. In performing this task, it also keeps track of non-toxigenic strains. Diphtheria is a notifiable disease in Belgium, and all potentially toxigenic *Corynebacterium* strains need to be referred to the NRC for confirmation, toxin detection and typing. In this overview, we present data on all registered cases of toxigenic *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* in Belgium during the past decade, up to and including 2017. We illustrate the importance of international efforts towards DAT-availability and vaccination, as well as underlining the need for continued and extensive diphtheria surveillance.

## METHODS

### Culture

The NRC accepts cultured isolates as well as clinical specimens from all of Belgium for *Corynebacterium* confirmation or identification. Clinical isolates and specimens – usually throat, nose or wound swabs – were processed on receipt by subculture on cystine tellurite blood agar (in-house) as well

as sheep blood agar (Oxoid Limited, Hampshire, UK) and incubated for 3 days at 35 °C under aerobic conditions at high CO<sub>2</sub> levels (5 %).

### Identification

Colonies showing haemolysis on sheep blood agar and grey-black colonies on cystine tellurite blood agar were identified using MALDI-TOF MS (Bruker, Massachusetts, USA). API Coryne (bioMérieux, Marcy l'Etoile, France) was used in case of MALDI-TOF scores below 2.000 and for biovar determination of *C. diphtheriae*.

### Toxin gene detection and confirmation of toxin production

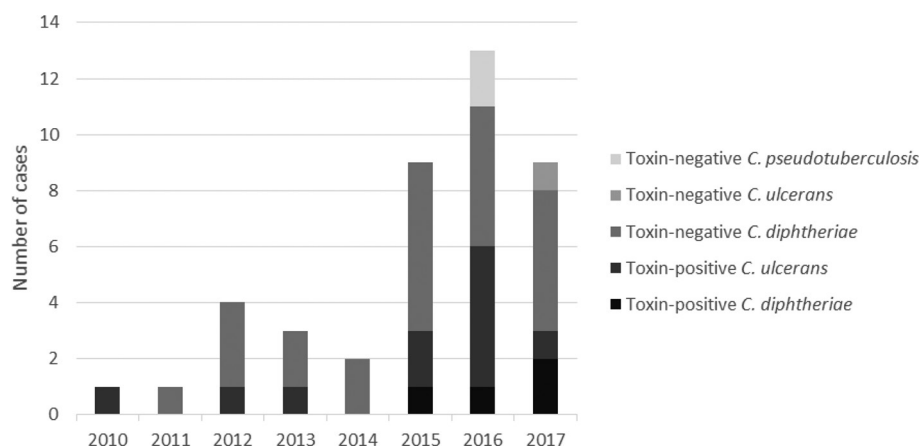
The presence of the diphtheria toxin gene (*tox*) was evaluated using a conventional PCR on heat-inactivated suspensions from all isolated *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. A duplex PCR was used, targeting the *tox* gene (primers DT1-f': 5'-CGGTGATGGTGCTTCGCG and 5'-DT2: CGCGATTGGAAGCGGGGT, based on Hauser *et al.* [22] with slight modifications) as well as the RNA polymerase subunit β gene (*rpoB*) (primers *rpoB*-Fseq: 5'-CGWATGAACATYGGBCAGGT and *rpoB*-Rseq: 5'-CCATYTCRCCRAARCGCTG) [23] as an internal control found in all *Corynebacterium* spp. Most PCR-*tox*-positive strains (*n*=15 of 23 (65 %), some strains isolated from animals excluded) were also tested in the WHO Global Collaborating Centre for Diphtheria and Streptococcal Infections, Public Health England (PHE), for toxin gene detection using a quadruplex real-time PCR assay for identification of potentially toxigenic corynebacteria [24] and for diphtheria toxin expression using the modified Elek test [25].

### Susceptibility testing

Susceptibility of the strains to penicillin, erythromycin, clindamycin and rifampicin was determined using ETEST (bioMérieux, Marcy l'Etoile, France), on Mueller-Hinton agar plates with 5 % horse blood and 20 mg l<sup>-1</sup> β-NAD (bioMérieux, Marcy l'Etoile, France), according to both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines [26, 27].

### Typing

Multilocus sequence typing (MLST) was performed on most strains (*n*=39 of 54 [72 %], including all toxigenic strains derived from human patients), as described in Bolt *et al.* [3, 28], with modifications to some primers (*leuA*-F2 : 5'-CGTRCACTTCTACAAMTC; *leuA*-R2 : 5'-RCCGTGRTCGGTCTTCAT). Sequence and allele types were assigned using the *C. diphtheriae* MLST database (<https://pubmlst.org/cdiphtheriae/>) developed by Keith Jolley and sited at the University of Oxford (Jolley and Maiden 2010) [29].



**Fig. 1.** Cases of *Corynebacterium* infection in Belgium since 2010 ( $n=43$ ). Number of registered *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* infections in humans in Belgium, yearly from 2010 until 2017.

## RESULTS

### Identification and toxigenicity

From 1990 until 2012, no cases of diphtheria were declared in Belgium. In 2012, a toxigenic *C. ulcerans* strain was found in the leg ulcer of an elderly woman living in poor hygienic conditions [30]. In 2013, an adapted PCR protocol was implemented (currently used) and strains were tested retrospectively, showing that a *C. ulcerans* strain from 2010, which had been considered non-toxigenic at that time, did contain and express the *tox* gene, making it the first registered case of diphtheria in Belgium since 1990. This was confirmed by PHE using the modified Elek test. No clinical data are available for this strain cultured from a leg ulcer.

The number of toxin-negative as well as toxin-positive *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* cases in human patients since its reoccurrence is shown in Fig. 1. Since 2010, 14 cases of toxigenic *Corynebacterium* infection were reported in human patients, ten *C. ulcerans* and four *C. diphtheriae*. A fifteenth toxigenic *C. ulcerans* isolate was found in a healthy carrier, a nurse of one of the patients. All relevant case-information on these 15 human toxigenic corynebacteria infections and related animal cases is summarized in Table 1 and further discussed below. Complete data on all registered corynebacteria strains found in Belgium during the past decade, including those found in animal screenings, are presented in Table S1 (available in the online version of this article) ( $n=54$ , of which 43 had a human source).

All strains isolated from primary human patients and found to carry the *tox* gene by PCR ( $n=14$ ) underwent toxin-production testing. All were found to produce the diphtheria toxin. However, one *tox*-bearing strain isolated from a cat was found to be non-toxin-producing [30]. Thus this strain was a non-toxigenic *tox*-bearing (NTTB) strain of *C. diphtheriae* [31, 32]. The PCR results from the Belgian

NRC and PHE showed complete concordance (15/15, 100 %).

### Infection sites

Out of the 15 toxigenic corynebacteria isolated in humans, 11 were *C. ulcerans*. Seven out of eleven *C. ulcerans* strains were isolated from ulcers or skin wounds (usually of the lower limb, one non-specified). A nurse caring for one of these patients was found to be a healthy carrier of a toxigenic *C. ulcerans* strain of the same sequence type (ST), after throat swab screening. The remaining three *C. ulcerans* infections were cases of respiratory diphtheria.

In 2015, the first recent strain of toxigenic *C. diphtheriae* was found in the leg ulcer of a partially vaccinated 54-year-old man who had recently travelled to Mecca, Saudi Arabia. Since then, two more cases of cutaneous diphtheria caused by *C. diphtheriae* were reported, as well as one fatal case of respiratory diphtheria in an unvaccinated three-year-old child of Chechen origin born in Belgium.

In total, six toxigenic *C. ulcerans* and three toxigenic *C. diphtheriae* strains were found in lower limb lesions (Table 1). In three of these cases the patient was confirmed diabetic, and at least two of the wounds were colonized by pathogens other than *Corynebacterium* spp., such as *Staphylococcus aureus*.

### Vaccination status

The 2016 fatal *C. diphtheriae* case is the only one where the vaccination status of the patient is known to have been completely unvaccinated. For most other patients, vaccination data are missing or incomplete, as is information regarding animal contacts.

**Table 1.** Cases of toxigenic *Corynebacterium* infection in Belgium from 2010 until 2017 (n=14)

Related animal and healthy carrier cases are also shown (n=6), all related cases are highlighted in the same colour. UNK: unknown, F: female, M: male, N/A: not applicable, Y: yes, N: no.

Strain ID	Collection date	Species	Host	Age (yr)	Gender	Vaccination status	Probable region of infection	Animal contact	Hospitalization	Site	ST	Clinical manifestation
DIFT028	06/10/2010	<i>C. ulcerans</i>	human	UNK	UNK	UNK	UNK	UNK	UNK	lower limb lesion	332	lower limb lesion
DIFT029	03/04/2013	<i>C. ulcerans</i>	human	61	F	UNK	Belgium	cats, dogs	Y	sputum	332	respiratory infection without fever or pseudomembrane
DIFT027	10/05/2013	<i>C. ulcerans</i>	cat	UNK	M	UNK	Belgium	N/A	N/A	nose/sinus	332	none, pet of patient DIFT029
DIFT026	10/05/2013	<i>C. ulcerans</i>	cat	UNK	M	UNK	Belgium	N/A	N/A	nose/sinus	332	none, pet of patient DIFT029
DIFT025	10/05/2013	<i>C. ulcerans</i>	cat	UNK	M	UNK	Belgium	N/A	N/A	nose/sinus	332	none, pet of patient DIFT029
DIFT024	10/05/2013	<i>C. ulcerans</i>	dog	UNK	M	UNK	Belgium	N/A	N/A	nose/sinus	332	none, pet of patient DIFT029
DIFT033	12/02/2015	<i>C. ulcerans</i>	human	84	F	UNK	Belgium	cows	Y	skin lesion	328	skin lesion caused by burn
DIFT036	16/05/2015	<i>C. ulcerans</i>	human	69	F	UNK	Belgium	UNK	UNK	lower limb lesion	331	lower limb lesion
DIFT046	13/04/2016	<i>C. ulcerans</i>	human	66	M	UNK	Belgium	cats, dogs	Y	throat	331	respiratory infection with pseudomembrane
DIFT049	27/06/2016	<i>C. ulcerans</i>	human	94	F	UNK	Belgium	N	Y	lower limb lesion	332	lower limb lesion
DIFT050*	04/07/2016	<i>C. ulcerans</i>	human	25	F	UNK	Belgium	N	N	throat	332	none, nurse of patient DIFT049 *healthy carrier
DIFT054	06/09/2016	<i>C. ulcerans</i>	human	56	F	UNK	Belgium	UNK	N	lower limb lesion	331	lower limb lesion
DIFT059	22/12/2016	<i>C. ulcerans</i>	human	57	M	UNK	UNK	UNK	N	lower limb lesion	331	lower limb lesion
DIFT067	06/07/2017	<i>C. ulcerans</i>	human	72	F	fully vaccinated	Belgium	dogs	Y	distal tracheotomy	328	respiratory infection with pseudomembrane
DIFT019	21/05/2012	<i>C. ulcerans</i>	human	72	F	fully vaccinated	Belgium	cats	UNK	lower limb lesion	328	lower limb lesion

Continued

Table 1. Continued

Strain ID	Collection date	Species	Host	Age (yr)	Gender	Vaccination status	Probable region of infection	Animal contact	Hospitalization	Site	ST	Clinical manifestation
DIFT020	11/06/2012	<i>C. diphtheriae</i> biovar <i>mitis</i>	cat	UNK	UNK	UNK	Belgium	N/A	N/A	nose/sinus	40	none, pet of patient DIFT019
DIFT040	07/10/2015	<i>C. diphtheriae</i> biovar <i>mitis</i>	human	54	M	partially vaccinated	Saudi Arabia (Mecca)	N	N	lower limb lesion	217	lower limb lesion
DIFT045	10/03/2016	<i>C. diphtheriae</i> biovar <i>gravis</i>	human	3	F	not vaccinated	Belgium	N	Y	throat	384	respiratory infection with pseudomembrane and cardiac complications
DIFT060	05/01/2017	<i>C. diphtheriae</i> biovar <i>mitis</i>	human	32	M	UNK	UNK	UNK	N	lower limb lesion	67	lower limb lesion
DIFT070	29/08/2017	<i>C. diphtheriae</i> biovar <i>mitis</i>	human	13	M	UNK	Cameroon or Belgium	UNK	N	lower limb lesion	510	lower limb lesion

F, female; M, male; N, no; N/A, not applicable; UNK, unknown; Y, yes.



## Country of infection

More data are known on the suspected country of infection. Nine out of fourteen toxigenic infections (eight *C. ulcerans* and one *C. diphtheriae*) were probably acquired in Belgium as recent travel (or contact with persons who had recently travelled) was specifically denied. The same is true for the only healthy toxigenic *C. ulcerans* carrier. One infection (*C. diphtheriae* in a lower limb lesion) may have been acquired in Cameroon as recent travel there was declared, but no further details were given. Another *C. diphtheriae*-infection of the lower limb was, as mentioned earlier, very likely acquired in Saudi Arabia as the wound was inflicted there.

## Patient age

Only two toxigenic *C. diphtheriae* infections occurred in children (a 3-year-old and a 13-year-old), the two others occurred in adults (age 32 and 54 years). All human toxigenic *C. ulcerans* infections occurred in adults (aged 56–94 years, median age 69 years), not including one case of unknown age and the 25-year-old healthy carrier.

## MLST

MLST was performed for most strains ( $n=39$  of 54 [72 %]), including all toxigenic strains found in humans ( $n=15$ ). The results (Table 1) showed that strains generally had different STs unless they were part of the same outbreak investigation. In total, 26 unique STs were found. Out of the 15 toxigenic strains found in humans, only seven different STs were found. Of those, the four *C. diphtheriae* strains all had different STs. Among the *C. ulcerans* strains, identical STs were sometimes found; two unrelated strains isolated from dogs had ST325, three unrelated strains from human patients had ST328, four unrelated strains from human patients had ST331, and ST332 was shared by strains isolated from a human patient and a healthy contact, an unrelated human patient and their four pets, and a third unrelated human patient.

## Susceptibility testing

All toxigenic and most non-toxigenic strains, including those found in animals, underwent susceptibility testing

for penicillin, erythromycin, clindamycin and rifampicin ( $n=45$ ). Table 2 presents MIC50 values, MIC90 values and percentages of susceptible or intermediate strains according to EUCAST and CLSI. No significant difference was observed between *C. diphtheriae* and *C. ulcerans* or toxigenic and non-toxigenic strains (Table S1). According to both EUCAST and CLSI, only 16 % of strains were susceptible to penicillin. Most other strains were classified as intermediate for the latter, while one strain with a MIC of 8 mg l<sup>-1</sup> was resistant. Break-points for erythromycin are still in preparation by EUCAST, but 93 % were susceptible according to CLSI, three strains with MIC above 256 mg l<sup>-1</sup> being considered resistant. For clindamycin, 20 and 38 % of strains were susceptible and 38 and 66 % resistant or intermediate for EUCAST and CLSI, respectively. One strain presented a MIC value of 256 mg l<sup>-1</sup>, clearly out of the range. Finally, all strains but one (98 %) were susceptible to rifampicin for both institutes, the last one having a MIC of 0.38 mg l<sup>-1</sup>. One toxigenic *C. diphtheriae* strain showed clear resistance against penicillin, erythromycin and clindamycin.

## DISCUSSION

During the 20-year period from 1990 to 2009, Belgium remained diphtheria-free. Since 2010 however, sporadic cases of infection by toxigenic corynebacteria have started to reoccur, along with an increase in incidence of non-toxigenic *Corynebacterium* infection (Fig. 1). During the 2010–2015 period, toxigenic cases were caused by *C. ulcerans* only, however, since 2015, four cases of toxigenic *C. diphtheriae* infection have occurred. One of these resulted in fatal respiratory diphtheria in an unvaccinated 3-year-old child. This fatal outcome can most probably be attributed to the delayed administration of DAT, on the tenth day after onset of symptoms. At the time, DAT was not readily available in Belgium and was supplied from the Dutch National Institute for Public Health and the Environment [10, 33]. In cases of suspected respiratory diphtheria, treatment with DAT should be started as soon as possible, and preferably within 48 h after first symptoms [5].

**Table 2.** MIC values and percentages of susceptible or intermediate strains ( $n=45$ )

Shown are EUCAST and CLSI breakpoints, MIC50 and MIC90 values for all tested strains, as well as the percentages of strains that were susceptible or showed intermediate susceptibility according to EUCAST and CLSI guidelines. S: susceptible, I: intermediate.

	Breakpoints				EUCAST*		CLSI	
	EUCAST*	CLSI	MIC50	MIC90	S	S+I	S	S+I
Penicillin	≤0.12 and >0.12	≤0.12 and ≥4	0.25	0.5	16 %	98 %	16 %	98 %
Erythromycin	in preparation	≤0.5 and ≥2	0.047	0.094	in preparation	in preparation	93 %	93 %
Clindamycin	≤0.5 and >0.5	≤0.5 and ≥4	1.000	6.000	38 %	38 %	38 %	66 %
Rifampicin	≤0.06 and >0.5	≤1 and ≥4	0.002	0.003	98 %	100 %	98 %	100 %

\*EUCAST states that 'in an ongoing study, the preliminary results indicate that the current breakpoints for benzylpenicillin and rifampicin are not useful for *C. diphtheriae*'.

In this fatal case, the patient was known to be unvaccinated, but most other patients' vaccination data are unavailable. However, since vaccination coverage is high in Belgium, it can be assumed that (partial) vaccination contributed to the limited severity of some diphtheria cases. Other data, such as likely origin of the infection, remain largely unknown as well, making it difficult to hypothesize reasons for the perceived rise in diphtheria occurrence. Patients with toxigenic infections were mostly older, with only two toxigenic infections occurring in children. The majority of patients were over 50 years old. A shift in incidence towards older age groups has been noted in previous studies, and may be linked to waning immunity, as seroprevalence has been shown to diminish with age [15, 16, 20]. In 2006, the Belgian seroprotection rate was minimal (only 20 %) in the age category 55–59 years [20, 21]. Vaccination coverage and seroprevalence should be further studied in relation to disease incidence.

Whilst most toxigenic strains were found in skin lesions, usually of the lower limb, five were found in respiratory sites. At least four of these led to respiratory diphtheria, which is cause for concern as it is the most dangerous form and potentially lethal. Of the cases of lower limb infection, at least three patients were confirmed diabetic, and some of the skin lesions contained other pathogens besides *Corynebacterium* spp. (e.g. *S. aureus*), making it difficult to be certain that *Corynebacterium* was the causative pathogen, and not merely present as a commensal organism within the skin flora.

MLST alone cannot be used to prove transmission without an epidemiological link, since some cases were observed of *C. ulcerans* infections sharing the same ST but occurring far apart from each other in time and place. However, strains from patients in close contact sharing the same ST are likely related. In 2013, MLST analysis revealed the same ST supporting the epidemiologic relatedness of *C. ulcerans* strains found in a patient and her four pets (three cats and a dog). In 2016, toxigenic *C. ulcerans* strains with the same ST were found in a patient and her nurse, a healthy carrier, supporting a possible transmission from one to the other, though the primary source remains uncertain. The index patient had not had contact with animals or raw milk and had not recently left the country. While human-to-human transmission of *C. ulcerans* is rare, some cases of possible or probable transmission have been described [34].

STs were also compared to those found in other countries, as registered in the *C. diphtheriae* MLST database (<https://pubmlst.org/cdiphtheriae/>, consulted on 18 March 2019) [29]. ST325 and ST331, which we found in multiple strains, are also well-represented in the database (21 and 20 out of 738 cases respectively, mostly from France and Germany). ST325 is often found in strains isolated from cats and dogs. Two of our toxigenic cases were probably imported from another country: one from Saudi Arabia and one from Cameroon. No other cases from Saudi Arabia were registered in the PubMLST database. One case from Cameroon was registered, but it did not share the same ST (ST183 instead of ST510). It should be noted that the isolates submitted to the PubMLST

database represent only a subset of all cases, and information such as travel history is often missing.

Despite the apparent emergence of non-toxigenic *tox* gene-bearing *C. diphtheriae* strains in Europe [32], the one *C. diphtheriae* strain isolated from a cat in 2012 [30] (ST40) remains the only one isolated in Belgium to date.

Besides antitoxin treatment, antibiotic therapy remains important to interrupt toxin production and spread of the organism. Erythromycin and penicillin are the antibiotics of choice for the treatment of diphtheria [5, 35, 36]. However, using CLSI breakpoints, only 16 % of strains were labelled as susceptible to penicillin. EUCAST states that 'in an ongoing study, the preliminary results indicate that the current breakpoints for benzylpenicillin and rifampicin are not useful for *C. diphtheriae*' [26]. It is well known *C. diphtheriae* has only a moderate natural susceptibility to penicillin, which is used to evaluate its susceptibility to all beta-lactam antibiotics [37]. As MIC<sub>50</sub> and MIC<sub>90</sub> values obtained in this study are in the same range of those reported in older studies [38, 39], most of our strains should be considered susceptible, with the exception of one toxigenic *C. diphtheriae* var *mitis*, possibly acquired in Cameroon, presenting a MIC of 8 mg l<sup>-1</sup> that also showed high MIC values for erythromycin and clindamycin. Most strains were susceptible to erythromycin, but three of them, including the last one, were clearly resistant with MICs higher than 256 mg l<sup>-1</sup>. Although most strains remain susceptible, antibiotic susceptibility testing with MIC determination should be performed to detect at least high erythromycin resistance and penicillin MICs above 2 mg l<sup>-1</sup>, considered resistant by CLSI.

Our results show a potential re-emergence of diphtheria in Belgium, seemingly following a Europe-wide trend: sporadic cases occur all over Europe [2, 15, 16]. In some East-European countries, diphtheria still remains a public health problem, with 1515 cases reported between 1994 and 2014 [40]. In 2015, an unvaccinated child died of respiratory diphtheria in Spain. The child had received DAT-treatment with a 4-day delay [9]. A rise in *C. ulcerans* cases in particular has been noted in many European countries [16, 34, 41].

At present, it is difficult to state an exact cause of this comeback. Possible causes could be waning immunity, decreased vaccination in certain populations, or import from other countries, but unfortunately information on vaccination, travel and foreign contacts are often lacking. Travel and migration from endemic areas has been shown to be a factor in the re-introduction of mainly cutaneous diphtheria in Europe [42, 43]. Improved diagnosis due to better techniques and heightened surveillance may also play a role. The routine use of MALDI-TOF on isolates from throat and wound swabs has likely led to an increase in the identification of non-toxigenic corynebacteria, and potentially toxigenic bacteria in skin lesions.

These results highlight the importance of continued and heightened efforts towards diphtheria surveillance and vaccination, as well as the necessity of quick access to a



ready-to-use DAT stockpile [10, 33]. Furthermore, diphtheria awareness should be promoted amongst clinicians and primary caregivers, so that quick diagnosis may be possible and prompt treatment can be ensured. When referring or reporting potential cases of toxigenic corynebacteria to the NRC or other relevant instances, care should be taken to include all relevant information regarding the possible source of infection, such as travel and animal contacts, in order to facilitate epidemiological surveillance.

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#### Conflicts of interest

The authors declare there are no conflicts of interest.

#### Ethical statement

Epidemiological data were collected anonymously in the frame of Decision N° 2119/98/EC of the European Parliament and of the Council, concerning the epidemiological surveillance and control of communicable diseases in the Community, as completed by Decision N° 1082/2013/EU.

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