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Human Clinical Isolates of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* Collected in Canada from 1999 to 2003 but Not Fitting Reporting Criteria for Cases of Diphtheria

Leanne M. DeWinter,[1]* Kathryn A. Bernard,[1] and Marc G. Romney[2]

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada R3E 3R2,[1] and Division of Medical Microbiology, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada[2]

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A 5-year collection of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* human clinical isolates yielded nine isolates from blood cultures of patients with invasive infections, stressing the importance of *C. diphtheriae* as a serious blood-borne pathogen. Seven percent of *C. diphtheriae* and 100% of *C. ulcerans* isolates produced diphtheria toxin, demonstrating that toxigenic corynebacteria continue to circulate.

Two serological studies of healthy adult Canadian blood donors determined that roughly 20% had antibodies to diphtheria toxin below the accepted protective threshold (12, 21). When organized by age group, the 60-year and over age group was the least protected group, with 41.8% and 36.3% below the threshold in the two studies. Due to the fact that blood donors comprise a relatively healthier group than the general population, immunity among the general population would be expected to be lower (17). Furthermore, in industrialized countries, subpopulations of individuals refuse vaccination for religious, philosophical, or other reasons and thus are without protection against diphtheria and other vaccination-preventable diseases. Therefore, outbreaks of diphtheria could occur in susceptible, unvaccinated populations and among adults whose antibody level has dropped below the protective threshold.

Due to ongoing universal diphtheria vaccination programs in Canada, there have been no or a few cases of diphtheria meeting the criteria for notification per year since 1986 (4, 14). Notifiable cases of diphtheria in Canada include those from which *Corynebacterium diphtheriae* is isolated from an appropriate clinical specimen and those with a histopathological diagnosis of diphtheria (1, 12, 15). Despite the low incidence of notifiable diphtheria in Canada, numerous isolates of *C. diphtheriae* or *Corynebacterium ulcerans* continue to be recovered from patients seeking medical treatment for infections. The majority of these isolations are not notifiable since they do not meet case criteria for diphtheria.

The aim of this study was to characterize human clinical isolates of *C. diphtheriae* and *C. ulcerans* that did not meet the criteria for a notifiable case of diphtheria to determine whether there is a potential reservoir of toxigenic organisms in Canada.

(The data were presented in part at the 8th European Laboratory Working Group on Diphtheria/Diphtheria Surveillance Network meeting in Copenhagen, Denmark, 16 to 18 June 2004.)

All isolates were human clinical isolates referred to the National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, between 1999 and 2003. A standard panel of conventional biochemical tests which included fermentation of sugars, amino acid decarboxylases, and other reactions was performed as outlined previously (2). Production of diphtheria toxin was assessed by the modified Elek test (7). Carriage of the entire diphtheria toxin gene, *tox*, and the *toxA* fragment of the gene was assessed by the PCR as previously described (6, 13). Isolates were assigned to biotypes based on biochemical characteristics as described previously (8).

By conventional methods, *C. diphtheriae* isolates demonstrated phenotypic characteristics consistent with those reported in the literature (8), including fermentation of glucose, maltose, and fructose but not xylose, mannitol, lactose, or sucrose. All isolates were urease negative; citrate utilization negative; esculin and bile esculin negative; lysine, arginine, and ornithine decarboxylase negative; and Voges-Proskauer negative.

Six of 89 (7%) isolates of *C. diphtheriae* produced diphtheria toxin and harbored the diphtheria toxin gene (Table 1). A further 6 of 89 (7%) isolates carried the entire *tox* gene but did not express it, and 2 of 89 (2%) isolates carried the *toxA* fragment of the gene only. *C. diphtheriae* biotype *mitis* isolates carried the full *tox* gene, whereas *C. diphtheriae* biotype *belfanti* isolates tested positive for the *toxA* fragment only. Among *C. ulcerans* isolates, three of three isolates (100%) produced diphtheria toxin, which were also detected by PCR. Table 1 summarizes the biotype/species and toxigenicity status of all isolates between 1999 and 2003. By far the most commonly received isolates were nontoxigenic *C. diphtheriae* biotype *mitis*, 54/89 (61%) and *C. diphtheriae* biotype *gravis* 24/89 (27%). Among the biotypes, there were more toxigenic *C. diphtheriae* biotype *gravis* than *C. diphtheriae* biotype *mitis*, with rates of 4/28 (14%) and 2/56 (4%), respectively. Thus, over a 5-year

* Corresponding author. Mailing address: National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba, Canada R3E 3R2. Phone: (204) 789-2137. Fax: (204) 789-5009. E-mail: leanne_dewinter@phac-aspc.gc.ca.

TABLE 1. Toxigenicity and isolation sites of referrals of *C. diphtheriae* and *C. ulcerans* in Canada between 1999 and 2003

Parameter	No. of isolates				
	C. diphtheriae			*C. ulcerans* (n = 3)	All isolates (n = 92)
	Biotype *mitis* (n = 56)	Biotype *gravis* (n = 28)	Biotype *belfanti* (n = 5)		
Produced diphtheria toxin	2	4	0	3	9
Carried diphtheria toxin gene[a]	6	0	2[b]	0	8
Nontoxigenic	48	24	3	0	75
Isolation site					
Blood	8	1	0	0	9
Other sterile site[c]	4	0	0	0	4
Ear	5	11	0	0	16
Throat	1	2	0	0	3
Sinus	0	0	3	0	3
Other nonsterile site[d]	35	14	2	3	54
Not stated	3	0	0	0	3

[a] Refers to isolates that carried the *tox* gene and did not produce diphtheria toxin.
[b] *C. diphtheriae* biotype *belfanti* carried the *toxA* fragment only.
[c] Other sterile sites included lungs and eyes.
[d] Other nonsterile sites included skin wounds (ulcer, abscess, burn) from numerous body parts (mostly hands, arms, feet, and legs), and a peritoneal catheter exit site.

period, there was an overall rate of 10% (9/92) toxigenicity among all isolates and a further 9% (8/92) of isolates carried the toxin genes but did not express them. None of the nonnotifiable referrals were identified as *C. pseudotuberculosis*, *C. diphtheriae* biotype *intermedius*, toxigenic *C. diphtheriae* biotype *belfanti*, or nontoxigenic *C. ulcerans* during this period.

The most common sources of *C. diphtheriae* and *C. ulcerans* were nonsterile sites, including skin and wounds, representing 59% (54/92) of all isolates, while 17% (16/92) were isolated from ears (Table 1). When examined by biotype, a different picture emerges; 39% (11/28) of all *C. diphtheriae* biotype *gravis* isolates and 9% (5/56) of *C. diphtheriae* biotype *mitis* isolates came from ears. *C. diphtheriae* biotypes *mitis* and *gravis* (all nontoxigenic) were isolated from throats at the low incidence of 2% (1/56) among biotype *mitis* isolates and 7% (2/28) among biotype *gravis* isolates. The total percentage of throat isolates from all specimen sites was only 3% (3/92). Three of four *C. diphtheriae* biotype *belfanti* isolates were from sinuses, and they were the only biotype isolated from sinuses.

Nine of 92 (10%) *C. diphtheriae* isolates were isolated from blood, including eight nontoxigenic biotype *mitis* isolates and one toxigenic biotype *gravis* isolate. All nine isolates were isolated from patients in the Vancouver area and were fairly spaced out over a 5-year period. Four of the nine patients died following the infection, though the patient who was infected with the toxigenic *C. diphtheriae* biotype *gravis* survived. Underlying medical and social conditions that appeared to contribute to the development of bacteremia included intravenous drug use, diabetes mellitus, homelessness, and skin colonization with *C. diphtheriae*. Most, but not all, of the patients lived in or frequented the impoverished skid row area of Vancouver, which was associated with diphtheria outbreaks up to and during the 1970s (3). This cluster of invasive isolates is consistent with reports from around the world of nontoxigenic invasive *C. diphtheriae* infections associated with known risk factors (9–11, 16, 18) and highlights the importance of considering *C. diphtheriae* a serious pathogen when isolated from blood.

There have been increasing reports of *C. ulcerans* human infections in the literature in which toxigenic strains have been responsible for classical pharyngeal and cutaneous diphtheria as well as being associated with other infections (5, 19, 20). Although only three isolates of *C. ulcerans* have been referred to the national reference center between 1999 and 2003, all three isolates were toxigenic. Disease caused by all diphtheria toxin-producing species of *Corynebacterium*, including *C. pseudotuberculosis* and *C. ulcerans*, must be considered in the differential of causative agents of diphtheria. In the United Kingdom, isolation of any toxigenic corynebacteria, including *C. pseudotuberculosis* and *C. ulcerans*, requires notification of local and national communicable disease control agencies (5).

Although universal vaccination has resulted in a very low incidence of diphtheria in Canada, human clinical isolates which harbor and produce the diphtheria toxin remain in circulation. Between 1999 and 2003, 16% (14 of 89) of referred *C. diphtheriae* isolates produced the diphtheria toxin or harbored the diphtheria toxin gene without expressing it; 100% of referred *C. ulcerans* isolates produced the diphtheria toxin. The continued circulation of toxigenic strains of *C. diphtheriae* and *C. ulcerans* highlights the importance of continuing vaccination programs against diphtheria.

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