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, Kathryn A. Bernard, and Marc G. Romney

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Received 23 December 2004/Returned for modification 8 February 2005/Accepted 28 February 2005

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
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 nontoxicogenic C. ulcerans during this period.

The most common sources of C. diphtheriae and C. ulcerans were nonsterile sites, including skin and wounds, representing 39% (54/22) 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 nontoxicogenic) 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 nontoxicogenic biotype mitis isolates and one toxicogenic 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 during this period.

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 toxicogenic 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.

We acknowledge Carol Shaw of the British Columbia Centre for Disease Control for obtaining isolate information. We also thank Deborah Wiebe and Emma Ongsansoy for excellent technical assistance.

REFERENCES


---

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C. diphtheriae</th>
<th>C. ulcerans</th>
<th>All isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biotype mitis</td>
<td>Biotype gravis</td>
<td>Biotype belfanti</td>
</tr>
<tr>
<td>Produced diphtheria toxin</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Carried diphtheria toxin gene*</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nontoxicogenic</td>
<td>48</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Isolation site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other sterile site*</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ear</td>
<td>5</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Throat</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sinus</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Other nonsterile site*</td>
<td>35</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Not stated</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Refers to isolates that carried the tox gene and did not produce diphtheria toxin.

*b* C. diphtheriae biotype belfanti carried the toxC 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.


