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Characterization of *Corynebacterium diphtheriae* Isolates from Infected Skin Lesions in the Northern Territory of Australia[▽]

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Corynebacterium diphtheriae **is commonly isolated from cutaneous skin lesions in the Northern Territory of Australia. We prospectively assessed 32 recent isolates from infected skin lesions, in addition to reviewing 192 isolates collected over 5 years for toxin status. No isolates carried the toxin gene. Toxigenic *C. diphtheriae* is now a rare occurrence in the Northern Territory.**

Corynebacterium diphtheriae is frequently isolated from cutaneous lesions in tropical areas, including the north of Australia and the central Australian desert region (2, 14), geographical areas that are part of the Northern Territory (NT). Toxigenic strains of *C. diphtheriae* are the causative agent of respiratory diphtheria, but strains of *C. diphtheriae* isolated from cutaneous lesions can be either toxigenic or nontoxigenic (18) and pathogens or colonizers. Cutaneous diphtheria, caused by toxigenic strains, can act as a reservoir for respiratory diphtheria (1, 8). Infections with nontoxigenic *C. diphtheriae* occur in patients subsisting in low socioeconomic conditions, regardless of the climate (10, 15, 18).

In the past, toxigenic *C. diphtheriae* has been isolated from patients living in the NT. In the central Australian desert region, 38% of *C. diphtheriae* isolates tested over a 7-year period starting in 1985 were found to be toxigenic by Elek testing (14). The last reported case of locally acquired diphtheria was a fatal respiratory adult case from the north of the NT in 1992 (2). Sporadic cases of cutaneous diphtheria have since occurred, but all have been acquired from outside Australia (2). For many years, standard laboratory practice in the NT has been to assess all *C. diphtheriae* isolates for toxin production; however, toxin-producing strains are seldom detected.

This study aimed to detect the current prevalence of toxigenic *C. diphtheriae* strains isolated from clinically infected skin lesions referred to the Royal Darwin Hospital (RDH) pathology department. The RDH pathology department serves the RDH and is a referral laboratory for the four public microbiology laboratories (Alice Springs [ASH], Katherine, Tennant Creek [TCH], and Gove [GDH]) in the NT, spread out over 1,300,000 square kilometers. In addition to using molecular methods at RDH to detect the diphtheria toxin-encoding gene (*tox*) in our more recent isolates, we retrospectively reviewed all *C. diphtheriae* isolates from clinically infected skin lesions, which had been referred from RDH or the four public microbiology laboratories to the Institute of Medical and Veterinary Sciences (IMVS), Adelaide, for *tox* testing over the last 5 years. The results of the study were used to evaluate the rationale for routine *C. diphtheriae* toxin testing in the NT.

C. diphtheriae isolates from clinical specimens submitted to the RDH pathology department were prospectively collected over a 5-month period (January to May 2011). Coryneform bacteria were screened using Oxoid tellurite agar (Hoyle's) and identified with the API (RAPID) Coryne system (bioMérieux, Marcy l'Etoile, France) (4, 20) if (i) isolated from normally sterile body sites, (ii) they were the predominant organisms from nonsterile clinical material, (iii) colony growth occurred outside the primary inoculum, or (iv) Gram-positive bacilli were seen in the direct staining. Other bacterial isolates were identified by conventional and automated microbiological methods.

C. diphtheriae cultures for toxin gene testing were resuspended to a 0.5 McFarland standard using 0.45% saline prior to DNA isolation using the MagNA Pure LC total NA isolation protocol as recommended by the manufacturer (Roche). Samples were subsequently tested for the presence of *tox* using a previously described PCR method (19). ATCC 13812 Park Williams 8, gravis, *tox*$^+$ (9), was used as the toxigenic positive control.

Thirty-two *C. diphtheriae* isolates were collected from clinically infected skin lesions. *C. diphtheriae* was not isolated from any throat specimens during this period. Microbiological features are shown in Table 1. Bacterial skin copathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Arcanobacterium haemolyticum*, were isolated from every specimen. *A. haemolyticum* (previously a member of the corynebacterium group, *Corynebacterium haemolyticum*) has been previously associated with cutaneous diphtheria isolates (10). Eighteen patients had three skin pathogens isolated: *C. diphtheriae*, *S. aureus*, and beta-hemolytic streptococci. The toxin gene was not detected in any of the *C. diphtheriae* isolates.

Two hundred and nineteen isolates of *C. diphtheriae* were obtained from clinically infected skin lesions over the period from 2005 to 2010; 197 were isolated at RDH, 18 were isolated at ASH, 3 were isolated at GDH, and 1 was isolated at TCH. Coryneform bacteria were identified as *C. diphtheriae* using the API (RAPID) Coryne system (bioMérieux, Marcy l'Etoile, France) (9). The biotypes were as follows: *C. diphtheriae* bv.

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TABLE 1. Microbiological characteristics of *Corynebacterium diphtheriae* ($n = 32$ isolates) isolated from clinically infected skin lesions

Characteristic	Value
Median age in yrs of patients (interquartile range)	38 (26–48)
No. of males (%)	19 (59)
No. of clinical specimens taken from:	
Lower limb	20
Upper limb	2
Trunk	4
Head and neck	4
Unknown	2
No. of specimens of biotype:	
C. diphtheriae bv. gravis	6
C. diphtheriae bv. mitis	7
C. diphtheriae bv. mitis/belfanti[b]	19
No. (%) of specimens showing coinfection with:	32 (100)
Staphylococcus aureus (methicillin sensitive)	17
Staphylococcus aureus (methicillin resistant[a])	7
Streptococcus pyogenes	22
Arcanobacterium haemolyticum	8
Gram-negative bacteria only[c]	11
Group G streptococci	3

[a] All methicillin-resistant *Staphylococcus aureus* isolates were community associated (21).

[b] The API (RAPID) Coryne system was not able to differentiate between *C. diphtheriae* bv. mitis and *C. diphtheriae* bv. belfanti.

[c] Gram-negative bacilli included coliforms ($n = 7$), mixed anaerobes ($n = 2$), *Proteus* species ($n = 1$), *Pseudomonas aeruginosa*, and *Aeromonas* species ($n = 1$).

mitis 127 (58%); *C. diphtheriae* bv. gravis 57 (26%); and ambiguous biotype, listed as *C. diphtheriae* 35 (16%). Over the same period, *C. diphtheriae* was isolated from blood cultures taken from 9 patients and was not isolated from any respiratory specimens. Of the 219 cutaneous *C. diphtheriae* isolates, *tox* testing of 192 isolates was negative. As clinical diphtheria becomes less frequent, laboratories should be aware of the potential for bacterial misidentification (16).

Corynebacterium diphtheriae is endemic in the NT and is regularly cultured from infected skin lesions, particularly from indigenous patients. Nontoxigenic *C. diphtheriae* can cause pharyngitis and invasive infection that may be fatal (i.e., pneumonia, sepsis, catheter-related infection, endocarditis, and osteomyelitis) (6, 21). Historical reports document that toxigenic *C. diphtheriae* was endemic in the central Australian desert region and the tropical north of Australia as late as the 1990s (2, 14), while diphtheria has been a rare occurrence in other Australian states following the introduction of the diphtheria-tetanus-polio vaccine in the 1950s (12). However, 224 *C. diphtheriae* isolates from infected skin lesions from both Central Australia and the tropical north of Australia, from the last 5.5 years, were all nontoxigenic. Our results suggest that toxigenic *C. diphtheriae* is now a rare occurrence in the NT.

The reasons for the reduction of the number of *tox*-carrying *C. diphtheriae* strains in the NT are unclear, although it has been postulated that *tox* may be lost from organisms in highly immunized populations (3). The toxin gene is carried on a bacteriophage, but the toxin is a nonessential protein for both the phage and its lysogenic host bacterium (13). The selective advantage arises in clinical diphtheria when the toxin allows rapid and sustained transmission of toxigenic organisms from nasopharyngeal membranes or cutaneous ulcers to nonimmune humans (13). Thus, widespread immunization protects against diphtheria and may also reduce transmission of toxigenic strains (13). The current diphtheria vaccination coverage in NT children at 2 years of age is 95.5% (11), and the indigenous rate is similar to the nonindigenous rate (7). A recent case of non-travel-associated diphtheria in a partially vaccinated teenager in the United Kingdom highlights the importance of continued vaccination (17). The use of penicillin in rheumatic heart disease and skin sore control programs in north and central Australian indigenous communities may also simultaneously treat cutaneous diphtheria, as well as eradicate the carrier state of toxigenic strains, thereby preventing transmission of toxigenic strains.

Although toxigenic *C. diphtheriae* was not isolated in our study, toxigenic strains continue to circulate in developing countries, including in South Asia, Southeast Asia, Africa, and parts of South America (22). Since 1992, the two cases of diphtheria in the NT have occurred in migrants or returned travelers from areas where *C. diphtheriae* is endemic, and pretravel vaccination is recommended (2). A high index of suspicion should be maintained for these high-risk groups, and diphtheria toxin testing of *C. diphtheriae* isolates should be performed. In addition, because *tox* is phage mediated, it is possible that toxigenic *C. diphtheriae* may reemerge (5) and spread locally if diphtheria immunization rates decline. Based on the findings of this study, our laboratory no longer tests for *C. diphtheriae* toxin routinely.

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We have no conflicts of interest.

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