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Canada's First Case of a Multidrug-Resistant *Corynebacterium diphtheriae* Strain, Isolated from a Skin Abscess▽

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A toxigenic *Corynebacterium diphtheriae* biovar mitis sequence type 136 (ST136) strain was recovered from a toe infection of an unvaccinated patient recently returned from India. The isolate was resistant to clindamycin, erythromycin (*ermX* positive), tetracycline, and trimethoprim-sulfamethoxazole, intermediate to ceftriaxone and cefotaxime, and had high MICs for telithromycin and chloramphenicol but was sensitive to other drugs.

CASE REPORT

A 38-year-old male was seen by a family physician in a city located in a western Canadian province for an evaluation of an abscess on his left second toe which started 3 days prior while the patient was visiting family and friends in India. The patient had no history of traumatic injury, denied contact with sick persons, and had an unknown vaccination history. A swab of the left second toe was sent within hours to a private laboratory for bacterial culture and drug sensitivity testing. The patient was prescribed 500 mg of cephalexin three times a day for 10 days, and he recovered uneventfully. Patient consent to describe this case was obtained for the purpose of this study.

The direct Gram stain of the specimen revealed Gram-positive cocci and Gram-positive bacilli. After ~48 h of incubation under facultatively anaerobic conditions at 35°C on 5% sheep blood agar, the culture grew colonies which were identified as group A streptococci and *Staphylococcus aureus*. In addition, the culture also grew creamy, opaque, slightly raised nonhemolytic colonies; Gram smear of the isolate revealed Gram-positive bacilli with club-shaped ends and occasional V forms. This strain was urease negative and facultatively anaerobic. Colonies were black with dark halos on Tinsdale medium (17). The isolate was referred to the BC Center for Disease Control Laboratory for confirmation and identification as *Corynebacterium diphtheriae*. This strain fermented glucose and maltose but not lactose, mannitol, glycogen, or xylose. When studied by conventional methods, this strain reduced nitrate to nitrite and was catalase positive. Black colonies typical for *C. diphtheriae* grew on freshly prepared cystine-tellurite blood agar (17). Albert's staining (18a) was performed after 24 h of growth on Loeffler's medium (PML Microbiologicals, bioMérieux), where typical blue-black metachromatic granules against a green cytoplasm were observed, consistent with *C. diphtheriae* (6). The strain was forwarded to the Canadian National Microbiology Laboratory (NML) for further characterization and toxigenicity testing (NML identifier 090066).

Growth in brain heart infusion broth was not enhanced by the addition of ~1% (vol/vol) sterile Tween 80, a feature that, if present, is suggestive of *C. diphtheriae* biovar intermedius (9). Using conventional carbohydrate broth sugars (2), the strain was corroborated as being positive for catalase, reduction of nitrate, and fermentation of glucose, fructose, galactose, maltose, mannose, and ribose but not glycerol, glycogen, lactose, mannitol, raffinose, sucrose, trehalose, or xylose. Oxidase was negative, and the isolate was nonmotile at 25°C and 35°C. The API Coryne strip (bioMérieux) generated a code of 1010324 with a high confidence value (95.9%) for *C. diphtheriae* biovar mitis/belfanti. Only α-glucosidase was detected using the API ZYM strip (bioMérieux). The isolate was also consistent for *C. diphtheriae* using cellular fatty acid composition analysis (1). PCR detection of the diphtheria *tox* gene was positive for both the 248-bp fragment and the complete *tox* gene (7, 13). The modified Elek test (8), used to determine production of the diphtheria toxin, was positive after 24 h of incubation. On the basis of these phenotypic and molecular findings, the isolate was confirmed as *C. diphtheriae* biovar mitis (toxigenic strain) (9). Multilocus sequence typing (MLST) of extracted DNA was done by PCR amplification of seven *C. diphtheriae* housekeeping loci (*atpA*, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA*, and *rpoB*) (3). Allelic numbers were assigned to each locus, creating a unique numerical profile, which was compared with *C. diphtheriae* sequences posted at http://pubmlst.org/cdiphtheriae/. The profile obtained was 3, 2, 4, 1, 3, 3, 13, which was assigned sequence type 136 (ST136), a type

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TABLE 1. Antibiotic susceptibilities of *C. diphtheriae* clinical isolate NML 090066

Antibiotics	Range tested (μg/ml)	MIC [μg/ml (interpretation[a])]
Cefotaxime	0.12–4.0	2.0 (I)
Ceftriaxone	0.12–2.0	2.0 (I)
Chloramphenicol[b]	1–32	>32
Clindamycin	0.12–2.0	>2.0 (R)
Erythromycin	0.25–4.0	2.0 (R)
Telithromycin[b]	0.5–4.0	>4
Tetracycline	1.0–16	16 (R)
Trimethoprim-sulfamethoxazole	0.5/9.5–4/76	>4/76 (R)
Cefepime	0.5–8.0	1.0 (S)
Ciprofloxacin	0.5–2.0	≤0.5 (S)
Daptomycin	0.06–8.0	0.12 (S)
Gentamicin	2.0–16	≤2.0 (S)
Linezolid	0.25–8.0	≤0.25 (S)
Meropenem	0.25–2.0	≤0.25 (S)
Penicillin	0.03–8.0	0.25 (S)
Quinupristin/dalfopristin	0.12–4.0	≤0.12 (S)
Rifampin	0.5–4.0	≤0.5 (S)
Vancomycin	0.5–128	≤1 (S)

[a] I, intermediate resistance; R, resistant; S, susceptible.
[b] Chloramphenicol and telithromycin MICs are not routinely reported (5) but were observed to be unusually elevated.

unique among NML data and, to date, from published literature (3, 11, 18).

Antimicrobial susceptibility testing (Table 1) was performed with the broth microdilution method using Mueller-Hinton medium containing 2.5% (vol/vol) lysed horse blood, commercial Sensititre STP5F and GNP3F plates (Trek Diagnostic), and interpretive criteria used as described in CLSI document M45-A2 (4, 5). The isolate was found to be resistant to clindamycin and erythromycin. The *ermX* gene, which is associated with this phenotype (15), was detected using methods described by Rosato et al. (16), but other resistance mechanisms were not studied. The isolate was also resistant to tetracycline and trimethoprim-sulfamethoxazole (TMP/SMX) but displayed MIC values for ceftriaxone and cefotaxime that fell into the intermediate category. Although no interpretation guidelines exist for telithromycin and chloramphenicol, the isolate demonstrated high MICs (μg/ml) of >4 and >32, respectively. The strain was sensitive toward penicillin, meropenem, cefepime, vancomycin, daptomycin, gentamicin, and linezolid. Penicillin and erythromycin are recommended as treatment for diphtheria (10).

Multidrug-resistant (MDR) *C. diphtheriae* strains have been recognized only very rarely in recent global literature. For instance, in Vietnam, 20% of isolates were found to be multiresistant to antibiotics when tested using disk diffusion and agar dilution methods (10). In a Brazilian study, 97% of *C. diphtheriae* strains were found to be resistant to between 4 and 7 antimicrobial drug classes using disk diffusion and Etest methods (14). In contrast, data collected from the Russian Federation outbreak of the early 1990s showed 2.4% monoresistance to trimethoprim and rifampin but no MDR (12) and, more recently, 0% of Polish strains (19) were found to be MDR. Contemporary data for MDR diphtheria isolates recovered in Canada or the United States remains scant.

This case report presents Canada's first-ever case of an MDR *C. diphtheriae* strain, acquired following recent travel to India. Subsequently, there has been no evidence of spread of this strain among close contacts. *C. diphtheriae* isolates referred to the Canadian federal reference center to date were sensitive to all antimicrobials tested using CLSI methods and broth microdilution methods, with rare exceptions of monoresistance or resistance to 2 drugs, including 7 strains resistant to erythromycin and clindamycin, linked to the presence of the *ermX* gene, and one strain resistant to tetracycline and TMP/SMX (T. V. Burdz, D. Wiebe, M. Walker, and K. Bernard, presented at the 108th Annual General Meeting of the American Society for Microbiology, Boston, MA, 1 to 5 June 2008; K. Bernard, unpublished data).

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