Three Variants of *Corynebacterium diphtheriae* subsp. *mitis* (Belfanti) Isolated from a Throat Specimen

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Three variants of a *Corynebacterium diphtheriae* subsp. *mitis* strain belfanti, two toxigenic (phage types 7 and 11) and one nontoxigenic (phage type 7), were simultaneously isolated from the throat of an adult.

Generally *Corynebacterium diphtheriae* subsp. *mitis* (belfanti) are not toxigenic (1). Gibson has reported that a nontoxigenic *C. diphtheriae*, isolated from a patient, later produced both toxigenic and nontoxigenic variants in the laboratory (5). Mixtures of toxigenic and nontoxigenic *C. diphtheriae* have been reported in skin lesions (2). This paper reports isolating, from a throat swab, a mixture of three different types of *C. diphtheriae* belfanti strains: two different toxigenic strains and one nontoxigenic strain.

On 29 March 1978, a 25-year-old woman was seen at the Outpatient Department, Toronto Western Hospital, for a hearing-aid assessment. Routine throat examination revealed a whitish secretion and small areas of heaped-up yellow membrane on the posterior pharyngeal wall. When the membrane was lifted with forceps, no bleeding points were uncovered. No ulceration was observed, and there was no odor. The patient had no fever and did not complain of a sore throat.

A swab of the throat was taken and the patient was sent home on ampicillin therapy. When colonies suggestive of *C. diphtheriae* were isolated, she was recalled, and on 1 April was given 40,000 U of diphtheria antitoxin and penicillin V, 300 µg four times a day for 10 days. The patient had never been immunized against diphtheria. Cultures were taken from home contacts (husband and three children) by Public Health authorities.

The patient's throat swab yielded a culture of *C. diphtheriae*, which was nitrate negative and toxigenic when tested in vivo by intradermal inoculation of a guinea pig (3) and in vitro by a modified Elek technique (4). Commercial KL virulence agar and enrichment (Difco Laboratories, Detroit, Mich.) were used with a strip of sterile filter paper (Whatman no. 1, 75 by 5 mm) saturated with 200 U of antitoxin (Connaught

Laboratories Ltd., Willowdale, Ontario) per ml. Because a toxigenic *C. diphtheriae* subsp. *mitis* (belfanti) is an unusual finding, further platings were carried out and tested (7). In all, three different types of *C. diphtheriae* were obtained. Although all were biochemically typical of *C. diphtheriae* subsp. *mitis* (belfanti), there were differences in cultural and microscopic morphology, toxigenicity, and bacteriophage type. One isolate, A, similar to the previously described culture, was soft, butyrous, hemolytic on 5% sheep blood agar, exhibited long slender gram-positive rods, and was phage type 7. A second isolate, B, which was quantitatively the predominant strain, resembled the first culture in all characteristics except that it was nontoxigenic. The third type, C, was toxigenic, friable, nonhemolytic, microscopically appeared shorter than A or B (Fig. 1), and was phage type 11. Table 1 presents a summary of the characteristics of these three cultures.

A throat swab taken from the patient on 26 April and throat swabs collected from the husband and two of their children were negative for *C. diphtheriae*. The third child (a 15-month-old boy) showed the presence of a nontoxigenic *C. diphtheriae* subsp. *mitis* (belfanti), phage type 7, similar to isolate B from the mother.

Despite their differences, the inability of the three variants to reduce nitrate suggests that they may be mutants of a single strain. The mechanism of the change in toxigenicity could be related to the interaction of lipid in the "cord-factor" (8), and the change in phage type could be related to the interaction of lipid in the phage acceptor at the cell surface (6).

Microbiologists must ensure that any culture of *C. diphtheriae* be thoroughly tested for toxigenicity. Physicians and Public Health workers should be aware of the possible coexistence of toxigenic and nontoxigenic variants when deal-

FIG. 1. Gram stain of 24-h growth on blood agar of *C. diphtheriae* subsp. *mitis* (*belfanti*) variants A, B, and C.

TABLE 1. Characteristics of three variants of *C. diphtheriae* subsp. *mitis* (*belfanti*) isolated from a single throat specimen

Culture	Toxigenicity	Phage type ^a	Remarks
A	Toxigenic	7	Soft, butyrous colonies, hemolytic, long slender rods
B	Nontoxigenic	7	Soft, butyrous colonies, hemolytic, long slender rods
C	Toxigenic	11	Friable colonies, nonhemolytic, short rods

^a Bacteriophage typing was carried out through the courtesy of C. H. Jellard, Provincial Laboratory of Public Health, The University of Alberta, Edmonton, Alberta, Canada.

ing with laboratory reports indicating nontoxigenic *C. diphtheriae*.

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