Diphtheria infections caused by the different toxigenic bio-
types of *Corynebacterium diphtheriae* have shown to be en-
demic in many parts of the world, including India (1, 7).
Among the total number of cases reported worldwide during
the year 2005, 80% of cases were from India (1). Diseases due
to nontoxigenic *C. diphtheriae* strains in vaccinated individuals
are now being increasingly reported globally (2, 9). However,
their incidence is very rare in India, as only one instance of
nontoxigenic strains causing diphtheria has been documented
earlier, from Pondicherry, India (6). We report here two more
similar cases from the same place with pharyngeal and naso-
pharyngeal etiology. The second such occurrence reveals that
nontoxigenic strains may be circulating in this region, thus
forming a potential reservoir.

DtxR is a global regulator involved in the regulation of
expression of diphtheria toxin, and nontoxigenic strains may
represent a potential reservoir for the emergence of toxigenic
strains if they possess functional *dtxR* and *tox* genes. Hence,
it is important to determine the carriage status of *tox* and *dtxR*
genes in our strains and to ascertain whether they are func-
tional. A comparison of *dtxR* sequences of our isolates with the
sequences of worldwide *dtxR* genotype strains was undertaken
to detect any similarity or other patterns. Sequence variations
in *dtxR* can serve as potential molecular subtyping markers for
the surveillance of the spread of particular strains across any
geographical area.

Of the two cases admitted during September 2008, the first
patient was suffering from high fever with throat pain and the
second one had fever with a history of nasal bleeding. Cultures
(IR74 and IR125) obtained from throat swab and membrane
from the tonsillar area of patients were identified as *C. diph-
theriae* biotype Gravis by standard biochemical tests. A toxigen-
icity test by Elek’s gel precipitation (3) was negative in both
cases. The first patient had been immunized earlier with a full
course of diphtheria-pertussis-tetanus (DPT) vaccine, whereas
the second one was not able to recollect the history of immu-
nization. Both patients recovered after antibiotic treatment
without any clinical complications.

*C. diphtheriae* strains were analyzed by PCR (Veriti; Applied
Biosystems), and direct sequencing was performed to determine
the presence and intactness of the *tox* and *dtxR* genes. PCR
primers were derived from earlier studies (2, 7, 8). Of the two
strains, only IR74 showed positive for the *tox* gene while both
were positive for *dtxR* amplification. Purified amplicons were
custom sequenced (Macrogen). Raw chromatography data were ed-
ited using GeneTool software and aligned using CLUSTAL W,
and the sequences were compared with previously published *dtxR*
sequences of *C. diphtheriae*, namely, NCTC13129, UK-NT95-407,
strain 1030, consensus sequence (2), Russian NTTB, Thailand,
Uzbekistan, PW8, and C7hm723 (2, 9). There was no correlation
between the PCR results and the Elek toxigenicity tests. Sequence
analysis of *dtxR* alleles showed that they were identical to the
published sequences of strain variants and found to be identical to
the majority of nontoxigenic strains of the United Kingdom
strain, the Russian NTTB strain, and an Uzbekistan strain and
also to the toxigenic PW8 strain. Amino acid sequence analysis of
the amplified *dtxR* genes from the two strains revealed variations
of the predicted DtxR protein. Our strains were identical with
variant 4, obtained in the United Kingdom (2), wherein there
were two amino acid changes (alanine to valine at residue 147 and
leucine to isoleucine at residue 214) compared with strain
NCTC13129.

An increase of systemic diseases due to nontoxigenic strains
has been observed recently in many parts of the world (9).
However, nontoxigenic diphtheria cases have not been re-
ported in India except in Pondicherry (5). Of the two nontox-
igenic strains studied, only one showed the presence of the *tox*
gene. Groman et al. (4) showed previously that nontoxigenic
strains can carry DNA sequences for the *tox* gene, whereby
such positive PCR cases were generally a consequence of am-
plification of DNA sequences that are part of a mutated or
partial, and therefore nonfunctional, *tox* gene. Data from our
study and other studies indicate that *tox*-bearing, nontoxigenic
*C. diphtheriae* strains are rarely isolated from human clinical
specimens (4, 6, 9).

Toxigenic strains have shown heterogeneity in sequences of
the toxin-regulatory element *dtxR*. Previously, it was shown
that mutations in the *dtxR* gene affect the functioning of DtxR
and that even a single amino acid substitution is capable of se-
verely diminishing or abolishing repressor activity (10). Hence,
it is essential to explore whether variation in *dtxR* has any
inhibitory effect on diphtheria toxin production. Investiga-
tion of our *dtxR* genotypes revealed that they were identical
to *dtxR* genotype variant 4 from the United Kingdom (2).
However, the exact association of *dtxR* variants with in-
creased or decreased toxin production is still unknown and
needs to be further evaluated. Nevertheless, one can specu-
late that naturally occurring variants of *dtxR* may be due to
point mutations having their association with increased or
decreased levels of toxin production and that such variations
may not necessarily result in amino acid substitutions.
Moreover, such variants can be potentially used for molec-
ular subtyping (8).

The source of our strains is unknown; however, a large
multicenter study involving large numbers of nontoxigenic
strains from different countries may be helpful in deducing the
source. The present and previous studies from Pondicherry
demonstrated the presence of nontoxigenic strains and their
potential to spread in this region. Although the limited number
of strains encountered may not enable us to draw any conclu-
sions, their persistence in this region is certainly evident. In
conclusion, diseases due to nontoxigenic strains of *C. diphthe-
riae* are least often reported in India, unlike the rest of the
world. Two nontoxigenic strains from Pondicherry reported
here were shown to be identical to global nontoxigenic *dtxR*
genotypes and the toxigenic PW8 strain.

**Nucleotide sequence accession numbers.** Nucleotide se-
quences of DtxR variants from strains IR74 and IR125 have
been deposited in GenBank under accession numbers
HM231328 and HM231329, respectively.

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