PERSISTENCE OF NON-TOXIGENIC CORYNEBACTERIUM DIPHTHERIAE BIOTYPE GRAVIS STRAINS IN PONDICHERRY, SOUTHERN INDIA

Diphtheria infections caused by the different toxigenic biotypes of Corynebacterium diphtheriae have shown to be endemic in many parts of world including India (1, 7). Among the total of 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 non-toxigenic 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 nasopharyngeal etiology. The second such incidence reveals that non-toxigenic 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 non-toxigenic 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 ascertain whether they are functional. A comparison of dtxR sequences of our isolates with the sequences of worldwide dtxR genotypes was undertaken to detect any similarity or otherwise. Sequence variations in dtxR can serve as potential molecular subtyping markers for the surveillance of the spread of particular strains across any geographical area.

Among 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 history of nasal bleeding. Cultures (IR74 25 & IR125) obtained from throat swab and membrane from tonsillar area of patients were identified
as *C. diphtheriae* biotype gravis by standard biochemical tests. Toxigenicity test by Elek’s gel precipitation (3) was negative in both the cases. The first patient had been immunized earlier with a full course of DPT whereas the second one was not able to recollect the past history of immunization. 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. PCR primers were derived from earlier studies (2, 7, 8). Of the two strains, only IR74 showed positive for tox gene while both were positive for dtxR amplification. Purified amplicons were custom sequenced (Macrogen). Raw chromatograph data were edited using GeneTool software, 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’s 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 majority of non-toxinogenic strains of UK, the Russian NTTB strain, an Uzbekistan strain and also with 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 UK (2) wherein there were two amino acid substitutions (alanine to valine at residue 147, leucine to isoleucine at residue 214) when compared with strain NCTC13129.

**Nucleotide sequence accession numbers:** Nucleotide sequences of DtxR variants from strains IR74 & IR125 have been deposited in GenBank under accession numbers HM231328 and HM231329 respectively.

Increase of systemic diseases due to non-toxigenic strains has been observed recently in many parts of world (9). However, non-toxigenic diphtheria cases have not been reported in India
except in Pondicherry (5). Among the two non-toxigenic strains studied only one showed the presence of \textit{tox} gene. Groman et al. (4) showed previously that non-toxigenic strains can carry DNA sequences for the \textit{tox} gene wherein such positive PCR cases were generally a consequence of amplification of DNA sequences that are part of a mutated or partial, and therefore nonfunctional, \textit{tox} gene. Data from our study and other studies indicate that \textit{tox}-bearing, nontoxigenic \textit{C. diphtheriae} strains are rarely isolated from human clinical specimens (4, 6, 9). Toxigenic strains have shown heterogeneity in sequences of toxin-regulatory element \textit{dtxR}. Previously it was shown that mutations in the \textit{dtxR} gene affect the functioning of DtxR and even a single amino acid substitution is capable of severely diminishing or abolishing repressor activity (10). Hence, it is essential to explore whether variation in \textit{dtxR} has any inhibitory effect on diphtheria toxin production. Investigation on our \textit{dtxR} genotypes revealed that they were identical to \textit{dtxR} genotype “variant 4” from the UK (2). However, exact association of \textit{dtxR} variants with the increased or decreased toxin production is still unknown and needs to be further evaluated. Nevertheless, one can speculate that naturally occurring variants of \textit{dtxR} may be due to point mutations having their association with increased or decreased levels of toxin production and such variations may not necessarily result in amino acid substitutions. Moreover, such variants can be potentially used for molecular subtyping (8).

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


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