Bacteriological & epidemiological characteristics of diphtheria cases in & around Delhi – A retrospective study

N.C. Sharma, J.N. Banavaliker, Rajesh Ranjan & Rajnish Kumar

Laboratory Department, Maharishi Valmiki Infectious Diseases Hospital, Delhi, India

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Background & objectives: Diphtheria infections caused by the different toxigenic biotypes of Corynebacterium diphtheriae are endemic in Delhi. Information on biochemical identification, toxigenicity and antimicrobial susceptibility to this bacterium is scanty. This retrospective study was carried out to identify isolated Corynebacteria biochemically, determine their toxigenicity, drug sensitivity and some epidemiological characteristics of diphtheria cases from Delhi and adjoining States for the period 1998-2004.

Methods: A total of 1118 throat and 585 nasal swabs were used to detect human pathogenic corynebacteria. WHO recommended methods were used for the detection, screening, toxigenicity and antibiogram pattern of the isolates.

Results: Among 493 (44.1%) cases detected positive for corynebacteria 71.8 per cent were pharyngeal, 20.9 per cent nasopharyngeal and rest 7.3 per cent nasal diphtheria cases. Biochemical identification revealed two species i.e., C. diphtheriae and C. pseudodiphtheriticum. In C. diphtheriae three biotypes were detected viz., intermedius (95.5%), gravis (3.4%) and mitis (1.1%). Toxin was expressed by 96 per cent isolates of C. diphtheriae. Cases were recorded from Delhi and four adjoining States. Sex ratio among male to female was 1.6:1. Prime victims were less than 9 yr old children (93.3%). Unvaccinated children (70.2%) were the main sufferers. Fatality rate was highest in Delhi cases (16.8%) followed by UP (14.6%) and Haryana (5.9%).

Interpretation & conclusions: Standard methods revealed the replacement of C. diphtheriae var mitis with var intermedius and occurrence of diphtheria infections due to other human pathogenic corynebacteria. It is imperative to have good bacteriological facilities to have better surveillance with regular monitoring in the endemic areas to keep the disease under control.

Key words Corynebacterium (a)-C. diphtheriae - C. pseudodiphtheriticum - cysteinase - gravis - intermedius - mitis - pharyngeal diphtheria - toxigenicity - vaccination

Pharyngeal or cutaneous diphtheria caused by toxigenic Corynebacterium diphtheriae and C. ulcerans remains a serious health problem in many regions of the world1-3. The epidemic in the Newly Independent States (NIS) of the former Soviet Union in 1990, the first major epidemic during the vaccination era, has revealed our incomplete understanding of the epidemiology of diphtheria, and the disease is endemic in many parts of
To the best of our knowledge bacteriological investigations were being done by the NICD, Delhi, for diphtheria patients admitted in Maharishi Valmiki Infectious Diseases Hospital (MVIDH), Delhi, even after 1983 but no information was available regarding the biochemical identification, toxigenicity and antibiotic sensitivity amongst the isolated strains. This may be due to rarity of cases, expenses and complexity associated with the laboratory diagnosis. Microbiological facilities are inadequate for quick diagnosis of diphtheria cases as has been realized by many workers. Keeping this in mind, laboratory facilities were developed in MVIDH, Delhi, which included isolation and biochemical identification of the isolates and testing of the toxigenicity as per standard guidelines. Moreover, the Laboratory department of MVIDH, Delhi, was selected under the National Surveillance Programme for Communicable Diseases (NSPCD) implemented amongst 100 districts in the country and has been reporting bacteriologically confirmed cases of diphtheria to the national nodal agency of NSPCD and Municipal Corporation of Delhi on monthly basis since June, 2003. In addition, the data have been analysed for age, sex, area, seasonality, vaccination status and case fatality during 1998-2004. We undertook this retrospective study to evaluate these

analysed data to understand the characteristics of diphtheria infections in this part of the country

**Material & Methods**

**Subjects:** A total of 1118 patients with clinical diagnosis of diphtheria were admitted in the Maharishi Valmiki Infectious Diseases Hospital, Delhi, during 1998-2004. Most of these patients were referred from the tertiary care facilities in Delhi and were labelled as probable cases.

**Inclusion criteria:** An illness of the upper respiratory tract characterized by laryngitis or pharyngitis and adherent membranes of tonsils, pharynx and nose. Isolation of *C. diphtheriae* from a clinical specimen was used to confirm the clinical diagnosis.

**Exclusion criteria:** Differential diagnoses were done for acute streptococcal membranous tonsillitis, infectious mononucleosis, non-bacterial membranous tonsillitis, primary herpetic tonsillitis, thrush and post tonsillectomy fualcial membranes in case of tonsillar and pharyngeal diphtheria. Presence of foreign body in the nose and rhinorrhea in cases of nasal diphtheria and infectious croup, spasmodic croup, epiglottitis and foreign body in the larynx.

**Detection of diphtheria organisms:** Clinical specimens (1118 throat swabs and 585 nasal swabs) were collected from the admitted patients for the detection of pathogenic corynebacteria following the standard procedures. The samples were taken into Amie’s charcoal medium (modified) and were kept at 4°C. These were sent to the laboratory within 24 h and were charged onto the plates of Columbia blood agar (Difco, Becton-Dickinson Company, USA) and Hoyles tellurite medium (Oxoid, England), both supplemented with 5% sheep blood and MacConkey Agar (BD, USA). Typical colonies growing on blood agar and Hoyles tellurite medium were selected after 24 and 48 h incubation respectively at 37°C and stained by Gram’s, Albert’s and Löeffler’s methylene blue stains.

**Cysteinease enzyme activity:** Morphologically confirmed colonies were screened for the presence of cysteinease enzyme activity using Tinsdale medium supplemented with Tinsdale supplement (Oxoid, England). At least 5 colonies from each culture were tested for the presence of enzyme at 37°C after incubation for 18 h. Colonies showing brown halo around the inoculum were selected for toxigenicity and biochemical identification.

**Biochemical identification of *C. diphtheriae*:** Biochemical identification was carried for 178 isolates
of corynebacteria according to current recommendations\(^{10}\). On the basis of morphology and cysteinase enzyme activity cultures were grown on sheep blood agar base No.2 (Oxoid, England) containing 5 per cent sheep blood at 37°C for 18 h. Growth was harvested and suspended into 3 ml suspension medium provided in the API Coryne Kits (Bio Merieux sa, France). Turbidity was adjusted to greater than 6 McFarland’s standard and the suspension was used to inoculate API Coryne strips. These were incubated at 37°C for 24 h. Species and biotype were determined using 20 characters supplemented with colony size and haemolysis using manual analytical profile index as per manufacturer’s instructions.

**Toxigenicity by Elek's method\(^{34}\):** Toxigenicity was determined in 176 diphtheria cultures as per the standard method\(^{30,33}\). *C. diphtheriae* CDC 510 and *C. diphtheriae* CDC 511 were used as control strains obtained from Dr S.S. Thukral, Department of Microbiology, V.P. Chest Institute, Delhi.

**Antibiotic sensitivity test:** Antimicrobial sensitivity of 54 isolates was carried out by disc diffusion method\(^{35}\) against 17 drugs (in µg) viz., amoxycillin/clavulanic acid (AMC-30), ampicillin (AM-10), ampicillin-sulbactum (SAM-20), azithromycin (AZ-15), cephotaxime (CTX-30), cephoxitin (FOX-30), ceftriaxone (CRO-30), cefuroxime sodium (CXM-30), chloramphenicol (C-30), ciprofloxacin (CIP-5), clindamycin (CC-2), doxycycline (D-30), erythromycin (E-15), gentamicin (GM-10), penicillin (P-10U), tetracycline (Te-30) and trimethoprim/sulphamethoxazole (SXT-1.25/23.75). Antibiotic discs (BD, USA) and diagnostic sensitivity test agar (DST) (Oxoid, England) supplemented with 5 per cent lysed sheep blood were used for antibiotic sensitivity testing (AST). Inoculum of each isolate was standardized to contain 10⁵-10⁶ organisms/ml using McFarland’s standard. *Staphylococcus aureus* ATCC 25923 was used as control strain. Results were recorded after incubation of plates at 37°C for 18-20 h.

**Characteristics of diphtheria infections:** To understand the trends of diphtheria infections the data of bacteriologically confirmed cases were analysed in terms of state, sex, age, seasonality, vaccination status and case fatality during the period under study.

**Results**

During the study period (1998-2004) the rate of detection of *C. diphtheriae* from probable cases increased from 26.3 per cent (45/171) in 1998 to 64.9 per cent (87/134) in 2004 (Fig. 1). Bacteriological positivity in terms of culture confirmed diphtheria cases was 44.1 per cent (493/1118) from 1118 throat swabs and 585 nasal swabs (Table I). 457 (40%) throat swabs and 139 (23%) nasal swabs were found positive for diphtheria organisms. Further analysis revealed 354/493 (71.8%) cases were positive in throat swabs only and labelled as pharyngeal, 103/493 (20.9%) nasopharyngeal having positivity in both throat and nasal swabs and 36/493 (7.3%) nasal diphtheria. Fatality rate amongst bacteriologically positive cases was 16.8 per cent in Delhi, 14.6 per cent in UP, 5.9 per cent in Haryana and 0.2 per cent each in Madhya Pradesh and Rajasthan with 37.7 per cent fatality during 1998-2004 (Table I).

Among 493 isolates, 491 (99.6%) were positive for cysteinase enzyme activity (Fig. 2a and 2b). A subset of 178 isolates subjected to biochemical identification revealed two species i.e., *C. diphtheriae* (176, 98.9%) and *C. pseudodiphtheriticum* (2, 1.1%) (Table II). Among *C. diphtheriae* isolates 168 (95.5%) were *C. diphtheriae* var intermedius, 6 (3.4%) were *C. diphtheriae* var gravis and 2 (1.1%) were *C. diphtheriae* var mitis. Of the 176 *C. diphtheriae* isolates, 169 (96.0%) tested positive for toxigenicity (Table II).

Of the 54 isolates tested for antibiotic sensitivity, 45 showed (83.3%) resistance to trimethoprim/
sulphamethoxazole followed by 18.5 per cent to ampicillin. Only one isolate was found resistant to ciprofloxacin. None of the isolates were multidrug resistant. All the 54 isolates were sensitive to rest of the 14 drugs tested.

Overall, 43.7 per cent cases were recorded from Delhi followed by 40.2 per cent from Uttar Pradesh (UP), 13.1 per cent from Haryana and rest from other States (Table I). Diphtheria cases occurred throughout the year particularly in 2004. Our data showed that 46 (9.3%) cases occurred from January to June whereas 447 (90.7%) cases were recorded from July to December during these years (Fig. 3).

Sex-wise distribution revealed more cases belonging to males (61.5%) as compared to females (38.5%) (Table III). In case of age-wise distribution, it was observed that cases in the age group 1-4 and 5-9 yr constituted majority of the patients i.e., 45.0 and 44.4 per cent. Parents/attendants were interviewed for vaccination status. Unvaccinated children were 70.2 per cent

Table I. Bacteriologically positive cases and fatality rate among diphtheria cases during 1998 - 2004

<table>
<thead>
<tr>
<th>States /UT</th>
<th>Years</th>
<th>C</th>
<th>B</th>
<th>D</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delhi</td>
<td>1998</td>
<td>97</td>
<td>26</td>
<td>12</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>65</td>
<td>17</td>
<td>10</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>63</td>
<td>18</td>
<td>4</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>64</td>
<td>17</td>
<td>9</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>62</td>
<td>18</td>
<td>13</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>86</td>
<td>38</td>
<td>13</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>52</td>
<td>27</td>
<td>10</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>489</td>
<td>206</td>
<td>83</td>
<td>778</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>43.7</td>
<td>41.8</td>
<td>16.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Uttar Pradesh (UP) C 49 | 50 | 57 | 45 | 79 | 109 | 60 | 449 | 40.2 |
| B 16 | 10 | 13 | 13 | 51 | 65  | 49 | 217 | 44.0 |
| D 4  | 2  | 7  | 3  | 16 | 25  | 15 | 72  | 14.6 |

Haryana C 22 | 16 | 26 | 22 | 20 | 21 | 19 | 146 | 13.1 |
| B 3  | 3  | 10 | 11 | 9  | 17  | 10 | 63  | 12.7 |
| D 3  | 1  | 5  | 6  | 3  | 6   | 5  | 29  | 5.9  |

Rajasthan C 2 | 2  | 2  | 5  | 4  | 14  | 3  | 2  | 1.3 |
| B 1  | 3  | 1  | 1  | 3  | 1   | 1  | 1   | 0.2 |
| D 1  | 1  | 1  | 6  | 1  | 1   | 1  | 1   | 0.2 |

Madhya Pradesh (MP) C 1 | - | - | - | - | 1   | 3  | 1   | 0.6 |
| B 8 | - | - | - | - | 1   | 3  | 1   | 0.2 |
| D 1 | - | - | - | - | 1   | 3  | 1   | 0.2 |

C, probable cases; B, bacteriologically positive cases; D, fatality
Denominator for C=1118 and denominator for B & D = 493
Three cases each from Bihar and Punjab were omitted from the Table since there was no positive case from these States.

Fig. 2. Eleven test isolates of C. diphtheriae showing brown halo around the growth (a) and individual colonies of one test strain (b) on Tinsdale medium.
**Table II.** Toxigenicity and biochemical identification of 178 *Corynebacteria* isolates during 1999-2004

<table>
<thead>
<tr>
<th>Species</th>
<th>Toxigenicity</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. diphtheriae</em> var intermedius</td>
<td>+</td>
<td>8</td>
<td>15</td>
<td>14</td>
<td>29</td>
<td>52</td>
<td>43</td>
<td>161</td>
</tr>
<tr>
<td><em>C. diphtheriae</em> var gravis</td>
<td>-</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td><em>C. diphtheriae</em> var mitis</td>
<td>+</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td><em>C. pseudodiphtheriticum</em></td>
<td>NT</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9</td>
<td>26</td>
<td>14</td>
<td>33</td>
<td>52</td>
<td>44</td>
<td>178</td>
</tr>
</tbody>
</table>

NT, not tested

**Table III.** Sex-wise, age-wise and vaccination status among bacteriologically positive diphtheria cases during 1998-2004

<table>
<thead>
<tr>
<th>Sex</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22</td>
<td>15</td>
<td>23</td>
<td>28</td>
<td>69</td>
<td>92</td>
<td>54</td>
<td>303</td>
<td>61.5</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>15</td>
<td>18</td>
<td>23</td>
<td>31</td>
<td>47</td>
<td>33</td>
<td>190</td>
<td>38.5</td>
</tr>
<tr>
<td>Age (yr):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>19</td>
<td>3.9</td>
</tr>
<tr>
<td>1-4</td>
<td>24</td>
<td>15</td>
<td>23</td>
<td>27</td>
<td>39</td>
<td>53</td>
<td>41</td>
<td>222</td>
<td>45.0</td>
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<tr>
<td>5-9</td>
<td>15</td>
<td>10</td>
<td>15</td>
<td>21</td>
<td>50</td>
<td>72</td>
<td>36</td>
<td>219</td>
<td>44.4</td>
</tr>
<tr>
<td>&gt;9 or above</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>33</td>
<td>6.7</td>
</tr>
<tr>
<td>Vaccination status:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Partially vaccinated</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>13</td>
<td>2.6</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>34</td>
<td>21</td>
<td>24</td>
<td>31</td>
<td>73</td>
<td>82</td>
<td>81</td>
<td>346</td>
<td>70.2</td>
</tr>
<tr>
<td>Unknown</td>
<td>6</td>
<td>6</td>
<td>13</td>
<td>16</td>
<td>23</td>
<td>53</td>
<td>2</td>
<td>119</td>
<td>24.1</td>
</tr>
</tbody>
</table>
followed by unknown group (24.1%). Partially vaccinated cases were 2.6 per cent followed by children belonging to vaccinated group (3%) (Table III).

Discussion

Bacteriological and epidemiological surveillance plays an important role in the control of diphtheria infections. C. diphtheriae infections remained firmly established in this part of the country. Rate of detection of diphtheria was 26.0 per cent in 1974 and declined to 4.1 per cent in 1981 whereas Singh et al. recorded 14.7 per cent cases in 1997. In the present study the rate of detection increased from 26.3 per cent in 1998 and reached 64.9 per cent in 2004 and was in agreement with other studies. In 8 families 2 or more cases were recorded. As both throat and nasal swabs were collected, 7.3 per cent cases could be labelled as nasal diphtheria leading to enhanced detection. Bacteriological positivity was reported only 7 per cent in a study conducted in a rural medical college of West Bengal, 12 per cent from Rajkot, Gujarat and 21 per cent from Chandigarh. In Russian epidemic microbiological positivity was 28 per cent and more than 89 per cent from Georgia and Russia respectively. C. diphtheriae var mitis was the main biotype followed by C. diphtheriae var gravis in this part of the country which has been replaced by C. diphtheriae var intermedius in our study. C. pseudodiphteriticum has also been reported earlier by some workers. In the present study two patients labelled as C. diphtheriae in routine diagnosis were later found to be suffering from C. pseudodiphteriticum.

Toxigenicity testing is considered an important criterion for notifying C. diphtheriae infections. About 70 per cent isolates from respiratory tract were reported to be toxigenic (1972 - 1974) by Ayyagari et al. and 46.7 per cent by Ray et al. as compared to the present 96.0 per cent toxigenicity among C. diphtheriae isolates. This was possible by adopting Elek’s improved method. Similarly, 75.5 to 100 per cent toxigenicity was demonstrated by other workers. Cysteinase enzyme test adopted for screening of human pathogenic Corynebacteria helped in rapid screening of these isolates.

Drug resistance to penicillin, chloramphenicol, erythromycin, tetracycline and ampicillin has been reported amongst the isolates from respiratory tract and from cutaneous lesions. These drugs had been included in the AST alongwith other drugs in clinical practice. All the 54 isolates tested were sensitive to 14 drugs including penicillin and erythromycin recommended in the diphtheria treatment.

Sex-wise ratio was 1.6:1 in male vs. female and remained the same in this part of the country whereas it was varying in different areas viz., 1.2:1 in Assam and Belgaum, 1.06:1 in Rajkot and 2.2:1 in Chandigarh which could be due to a bias towards the male child for seeking early treatment in the Indian society. Age-specific incidences were 93.3 per cent in <1, 1-4 and 5-9 yr and only 6.7 per cent in above 9 yr old patients. Minimum age of diphtheria patient was recorded to be 4 months. Shift to the age group of 5-9 yr has been observed. Similar findings were reported from other parts of the country and Belgaum which could be due to a bias towards the male child for seeking early treatment in the Indian society.

Immunization histories revealed unvaccinated group along with unknown group constituted 94.3 per cent children who did not receive vaccination whereas it was 59.5 per cent in an earlier study. Lodha et al. reported four unvaccinated cases of diphtheria from a tertiary care hospital in Delhi. Our findings are in agreement to other workers. Only 3 per cent vaccinated children were found to suffer from mild disease without any fatality. In ad-hoc national and subnational surveys conducted by the WHO it was found that the real coverage was substantially lower and needed improvement in the vaccine delivery system. Observations regarding the seasonal incidences of the disease were similar to the earlier findings and other parts of the country.

Case fatality was reported 32 to 56.3 per cent in different centres in north India, 13.2 per cent in south India, 16 per cent in North Eastern State and 42.9 per cent in west India. Higher case fatality was attributed to non-availability of antitoxin by some workers in India and in Russia and Lithuania. The other possible reason suspected by Singh et al. was misdiagnosis and poor handling of the specimen in the peripheral areas which certainly affect the isolation of C. diphtheriae thereby establishing diphtheria infection bacteriologically. Additionally, population migration and overcrowded urban slums may be contributory factors for the occurrence of the disease.
In conclusion, the findings of our study showed that by adopting WHO recommended methods in the laboratory, cases of diphtheria can be identified rapidly and correctly. *C. diphtheriae* var intermedius was the main species identified during 1998-2004. Further, keeping in view the endemicity in this part of the country and uprizing reports of diphtheria infections in the country as a whole, there is an urgent need to improve the microbiological services by adopting modern laboratory methods, improve vaccination coverage in identified pockets, enhanced surveillance and maintain stocks of diphtheria antitoxin in district level hospitals to reduce the morbidity and mortality.

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References


Reprint requests: Dr N.C. Sharma, Bacteriologist, Department of Bacteriology, MVID Hospital, Kingsway Camp Delhi 110009, India
e-mail: sharmanc1@rediffmail.com