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Persistence of *Corynebacterium diphtheriae* in Delhi & National Capital Region (NCR)

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Despite the introduction of mass immunization, diphtheria continues to play a major role as a potentially lethal infectious disease in many countries. Delay in the specific therapy of diphtheria may result in death and, therefore, accurate diagnosis of diphtheria is imperative. This study was carried out at National Centre for Disease Control (NCDC), Delhi, India, on samples of suspected diphtheria cases referred from various government hospitals of Delhi and neighbouring areas during 2012-2014. Primary identification of *Corynebacterium diphtheriae* was done by standard culture, staining and biochemical tests followed by toxigenicity testing by Elek's test on samples positive for *C. diphtheriae*. The results showed persistence of toxigenic *C. diphtheriae* in our community indicating the possibility of inadequate immunization coverage.

Key words *Corynebacterium diphtheriae* - diphtheria toxin - Elek's test

Diphtheria is an infectious disease caused by the exotoxin produced by *Corynebacterium diphtheriae*[1]. Despite the success of mass immunization in many countries worldwide, diphtheria continues to play a major role as a lethal resurgent infectious disease. An accurate microbiological diagnosis of diphtheria is crucial and is always regarded as being complementary to clinical diagnosis, because diphtheria is often confused with other conditions, such as severe streptococcal sore throat, Vincent"s angina, or glandular fever[2]. Detection of the lethal and potent exotoxin produced by the causative organism is considered to be the definitive test for the diagnosis of toxigenic *C. diphtheriae*. Early diagnosis and timely intervention not only help to reduce the incidence of the disease but also in containing the infection in the community and decreasing morbidity and mortality in the affected individuals. The inadequacy of microbiological facilities for rapid diagnosis of diphtheria cases has been realized by many workers[3,4]. The present study was aimed to perform the primary identification of *C. diphtheriae* from suspected cases of diphtheria in and around Delhi, and to determine their toxin producing activity.

A total of 941 throat and/or nasal swab samples from clinically suspected cases of *C. diphtheriae* were received from various government hospitals of Delhi and National Capital Region (NCR) at the National Centre for Disease Control (NCDC), Delhi, India, from January 2012 to December 2014. All these samples were accompanied with duly filled proforma

having the demographic details of the patients. Amie's charcoal medium (Becton-Dickinson, USA) was provided to all the centers for transportation of the samples. The samples were transported in cold chain. Blood agar and Hoyle's tellurite medium (Oxoid, UK), both supplemented with five per cent sheep blood were used for cultivation of the bacteria. Identification of *C. diphtheriae* was done by standard methods. *In vitro* toxin production was determined in 68 cultures positive *C. diphtheriae* isolates by Elek's gel precipitation test using Elek's test medium prepared according to the protocol[5] and anti-diphtheritic serum was procured from Central Research Institute, Kasauli, Himachal Pradesh, India.

Of the 941 samples received, 218 (23.2%) were tested to be positive by culture for *C. diphtheriae* (Table). The percentage positivity of diphtheria cases in 2012, 2013 and 2014 were as 26.1, 30.6 and 17 per cent, respectively. The highest numbers of cases were obtained from Haryana (35%) followed by Uttar Pradesh (30%), Delhi (17%), Rajasthan (15%) and others (3%) during the study period. Majority of the samples were obtained from the rural areas of the respective States. Age-wise distribution of samples was (0-1 yr) 2.3 per cent, (>1-5 yr) 56 per cent, (>5-10 yr) 26.6 per cent and (>10 yr) 15.1 per cent. Maximum number of cases was found in the age group 1-5 yr. Of the 218 samples positive for *C. diphtheriae*, toxigenicity testing by Elek's test was done in randomly selected 68 positive samples, of which 60 (88.2 %) were positive for toxin production. The seasonal distribution of the disease showed that maximum numbers of the cases were reported in the month of September and October.

Our study highlights the persistence of diphtheria in Delhi and NCR from 2012 to 2014. Various States of India have reported the persistence and resurgence of diphtheria[6,7]. In 1980, the cases of diphtheria reported were 39,231 which were reduced to 2817 in 1997. However, the past two decades have again seen a sudden increase in diphtheria cases with more than 8000 cases reported in 2004[1]. According to WHO UNICEF 2012[8] estimates, the primary immunization coverage for diphtheria has remained between 56 to 72 per cent[8]. The DPT 3 (diphtheria, pertussis, tetanus) coverage during 2002-2013 was 58-72 per cent and DPT booster dose 41.4 per cent according to National Family Health Surveys (NFHS)[9]. This re-emergence of diphtheria in the country seems mainly due to low coverage of primary immunization as well as boosters doses[1]. The National Health Profiles released by the Government of India reported 3812, 3977, 3529, 3123, 3485 and 3902 cases of diphtheria, respectively from 2007 to 2012. Recent report of National Health Profiles released by the government of India in 2013 showed that the number of reported cases of diphtheria in Delhi was 349 in 2009 and 150 during 2013[10]. The reason for such persistence could be failure of Universal Immunization Programme (UIP) to achieve satisfactory vaccine coverage in their respective regions[11]. Toxigenicity was found in 88.2 per cent (60/68) isolates in the present study. Toxigenicity testing has been considered an important criterion for notifying *C. diphtheriae* infections. Ayyagari *et al*[12] reported about 70 per cent isolates of *C. diphtheriae* from respiratory tract to be toxigenic (1972-1974). Similarly, some other workers have reported 75.5 to 100 per cent toxigenicity in their study[13].

Diphtheria mainly affects children aged between 1 to 5 yr, however, due to good vaccine coverage worldwide, a shift in age incidence has been observed from preschool to school age (5-15 yr) with more and more cases now being reported in adults[3]. In our study, maximum cases were seen in the age group 1-5 yr raising the possibility of poor vaccination coverage. Further, most of the cases were found in rural areas of neighbouring States of Delhi. The reason for this could be logistic problems like insufficient supply of vaccines, poor clinic organization, non-availability of immunization services on all days of the week and inadequate screening for immunization status of children visiting the health facility. Low awareness among care givers about utility of vaccination, vaccination schedules and vaccine preventable diseases could be another contributory factor leading to poor immunization[14]. Additional factors that prevent completion of vaccination include ignorance about total doses required, no/improper counselling about next vaccination visit, loss of vaccination cards with incorrect recall about the child's immunization status and vaccine side effects[14].

The present study demonstrated the presence of toxigenic strains of *C. diphtheriae* among a substantial percentage of a bacteriologically confirmed cases of diphtheria. There is a need for continued surveillance for diphtheria in this part of the country and review the efficacy of immunization programme in the country.

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Table. Positivity of suspected cases of *C. diphtheriae* samples over three years period (January 2012 to December 2014)

Month	Year						Total	
	2012		2013		2014			
	Samples tested	Positive for *C. diphtheriae*	Samples tested	Positive for *C. diphtheriae*	Samples tested	Positive for *C. diphtheriae*	Samples tested	Positive for *C. diphtheriae*
January	10	2	5	1	6	0	21	3 (14.2)
February	9	2	6	1	0	0	15	3 (20)
March	3	1	8	3	00	0	11	4 (36.4)
April	1	0	6	0	03	0	10	0 (0)
May	4	2	2	1	02	0	8	3 (37.5)
June	3	0	7	0	12	0	22	0 (0)
July	4	0	6	2	09	1	19	3 (15.8)
August	19	1	31	12	57	7	107	20 (18.7)
September	75	23	69	21	140	26	284	70 (24.6)
October	72	20	70	31	78	32	220	83 (37.7)
November	31	10	37	07	71	5	139	22 (15.8)
December	14	3	18	02	53	2	85	7 (8.2)
Total	245	64 (26.1)	265	81 (30.6)	431	73 (17)	941	218 (23.2)

Figures in parentheses indicate percentages

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