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Case Series

Microbiology Section

Recent Outbreaks of Diphtheria in Dibrugarh District, Assam, India

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ABSTRACT

Diphtheria is still a significant child health problem in countries with low immunization coverage. Reports of diphtheria in adult population are also increasing. Here we describe three recent outbreaks of diphtheria in Dibrugarh district, Assam in two consecutive years. The study was undertaken in Assam Medical College & Hospital, Dibrugarh after the diagnosis of two Diphtheria cases in the month of September and October 2015 and another in January 2016. Outbreak investigation was done after defining operational definition and throat swabs were collected from thirty three (33) individuals including three (3) index cases and thirty (30) close contacts. Diagnosis was done by clinical findings, direct microscopy, bacteriological culture and in-house designed multiplex Polymerase Chain Reaction (PCR) of the isolates for the expression of *Corynebacterium diphtheriae* specific *rpoB* gene and *tox* gene. Out of the 10 confirmed cases, 2 and 7 were in the first two outbreaks while only one in the third outbreak respectively. All the cases were of age > 10 years, unimmunized or partially immunized.

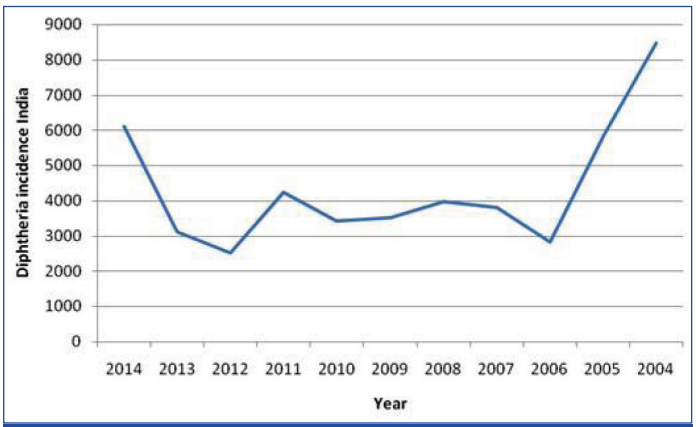
The overall mortality was 20%. PCR results revealed all the culture positive isolates to be *tox* gene positive. Diphtheria is a resurgent problem in our region with a significant age shift towards adult.

Keywords: *Corynebacterium diphtheriae*, Diphtheria antitoxin, Immunization, *Tox* gene

INTRODUCTION

Diphtheria is an acute, toxin-mediated, infectious disease caused by the bacterium *Corynebacterium diphtheriae*. Despite the Universal Immunization Programme of India, which offer 3 doses of the Diphtheria, Pertussis and Tetanus (DPT) vaccine starting at 6 weeks of age followed by 2 booster doses at 18 months and between 54 and 72 months of age, there have been a number of reports of either re-emergence or persistence of diphtheria from several Indian states including Assam in the last 5-10 years. The data on vaccine-preventable diseases provided by the Government of India to the World Health Organization indicates persistence of diphtheria without much decline over the last 10 years [1] [Table/Fig-1]. India alone accounted for 83.3% of the global burden of Diphtheria in 2014 [1].

National Health Profiles data of Government of India (CBHI, 2011) showed that the number of reported cases of diphtheria in Assam increased from 894 (no death) in 2011 to 1450 (no death) during 2013 while seventeen deaths reported out of 506 cases during the year 2014 [2]. Though frequent sporadic cases have been diagnosed in recent years, the last reported outbreak in Dibrugarh district of Assam was in 2009, where adults were primarily affected [3,4].



[Table/Fig-1]: Diphtheria incidence in India (2004-2014).

[Table/Fig-2]: Diphtheria outbreak locations, Dibrugarh district, Assam, India (Source: Google map).

Here we report three outbreaks [Table/Fig-2] in two consecutive years in Dibrugarh district of Assam, India.

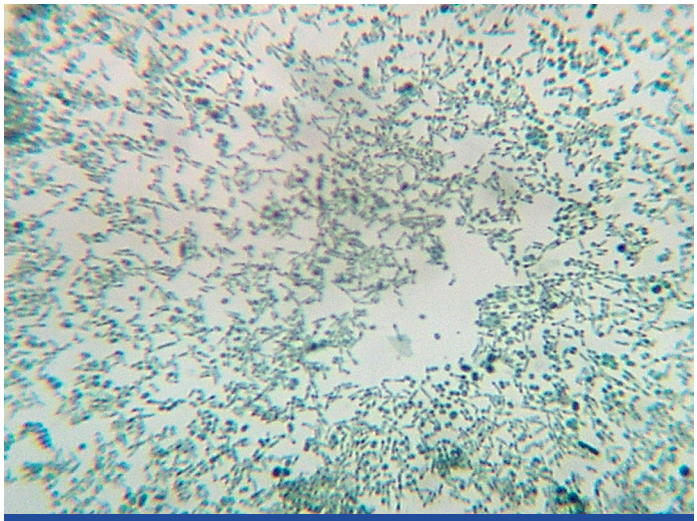
CASE SERIES

The present study involves a total number of thirty three (33) individuals which include one index case of each outbreak and 30 close contacts. The study was undertaken in Assam Medical College & Hospital, Dibrugarh, Assam, in the months of September and October, 2015 and January, 2016. The diagnosis of diphtheria infections was based on clinical manifestations with bacteriological confirmation by standard microbiological methods. Outbreak investigation was done with the use of the following operational definitions:

Index case: The first confirmed case or instance of a patient coming to the attention of health authorities.

Suspected case: Patient of any age with upper respiratory tract disease (pharynx, larynx, tonsils, nose), characterized by throat pain, mild fever, with adhering pharyngeal membrane and/or difficulty in swallowing or breathing.

Confirmed case: A symptomatic or asymptomatic case confirmed by isolation of toxigenic *Corynebacterium diphtheriae,* or



[Table/Fig-3]: Albert staining of Klebs Loeffler bacillus.

any suspected case that has an epidemiological link with a laboratory-confirmed case.

Contact: Any person living with a confirmed case, or frequenting his/her household or having some link (occupational, academic, or social) with a confirmed case.

All the culture positive isolates of *C. diphtheriae* using Sheep Blood Agar (SBA) and Potassium Tellutite Agar (PTA) were subjected to in-house designed multiplex PCR for the detection of *C.diphtheriae* specific *rpoB* gene (in house designed primer pairs: forward 5´-AGCTGGGCGACTCGGTTC-3´ and reverse 5´-GCTCAGAAA GAGACAGCGACATATT-3´) and *tox* gene (primer pairs: forward 5´-CGTACCACGGGACTAAACCT-3´ and reverse 5´-AGTGCGA GAACCTTCGTCAG-3´) yielding products of 105 base pair (bp) and 226 bp respectively.

Case 1

It is from outbreak 1. The index case, 13-year-old, unimmunized girl from Naliapool town of Dibrugarh district with fever, sore throat and membrane over the tonsils presented to ENT outdoor on 21st September, 2015. Direct microscopy for Klebs-Loeffler bacillus (KLB) by Albert staining [Table/Fig-3] and culture of throat swab on SBA and PTA confirmed presence of *C. diphtheriae*. Treatment started with erythromycin in isolation ward, diphtheria antitoxin was not administered due to unavailability. The patient showed initial improvement and the membrane had cleared off but later she lost her life on day 15 due to myocarditis.

During outbreak investigation, the contacts were found to be unimmunized and residing in an overcrowded housing complex with problem of poor ventilation. Only one out of four (1/4) close contacts was found culture positive for *C.diphtheriae* [Table/Fig-4].

Case 2

This case is from outbreak 2. The index case, 17-year-old unimmunized girl from Bagrodia Tea Estate, Tengakhat of Dibrugarh district was admitted in our hospital on 5th October, 2015 with complains of sore throat, fever and dysphagia with a thick whitish membrane covering both the anterior pillars and soft palate extending to the base of the tongue. Direct microscopy and culture confirmed presence of *C. diphtheriae*. Treatment was started with erythromycin, but diphtheria antitoxin was not available. She too lost her life on day 13 due to diphtheric myocarditis. All the close contacts were asymptomatic and unimmunized/ immunization status unknown. Six out of eleven (6/11) close contacts of this outbreak were found culture positive for *C. diphtheriae* [Table/Fig-4].

Case 3

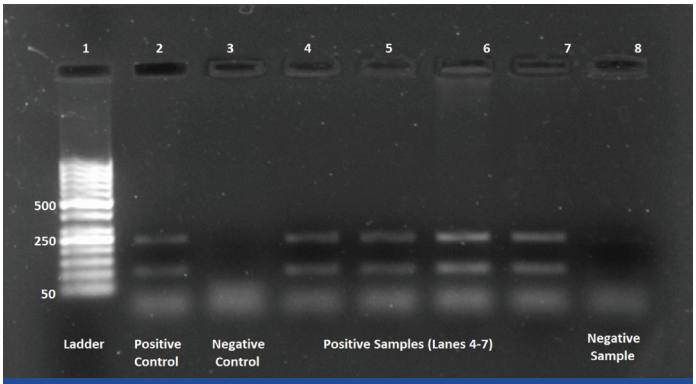
This case is from outbreak 3. The index case, an 18-year-old fully immunized boy from Haahorial village, Lahoal of Dibrugarh district reported to our hospital with pain in throat and a grey membrane over uvula without fever on 21st January, 2016. Direct microscopy and culture revealed presence of *C. diphtheriae.* Prompt isolation and treatment with diphtheria antitoxin and erythromycin was done. The membrane cleared off gradually and repeat culture was negative after 10 days. All the close contacts (15 numbers) were fully immunized, found asymptomatic and culture negative.

DISCUSSION

Diphtheria is an infectious disease caused by the exotoxin produced by *C.diphtheriae* which may cause obstructive pseudo-membranes in the upper respiratory tract or damage to myocardium and other tissues [5]. Although most infections are asymptomatic or run a

Outbreak No. & Location	Age (years)	Sex	Immunization status	Contact history	Symptoms and sign	Culture for *C. diphtheriae*	PCR for *rpoB* and *tox* gene for *C. diphtheriae*	Complication	Outcome
1 Naliapool, Dibrugarh	13	Female	unimmunized	Index case 1	Fever and sore throat for 3 days (membrane seen)	Positive	Positive	Myocarditis	Death (day 15)
	30	Female	unknown	Household contact of outbreak 1 (Mother)	Asymptomatic	Positive	Positive	-	Recovered
2 Bagrodia Tea Estate Tengakhat, Dibrugarh	17	Female	unimmunized	Index case 2	Sore throat and dysphagia for 5 days and fever for 3 days (membrane seen)	Positive	Positive	Myocarditis	Death (day 13)
	65	Male	unknown	Household contact of outbreak 2 (Father)	Asymptomatic	Positive	Positive	-	Recovered
	13	Male	unimmunized	Household contact of outbreak 2 (Brother)	Asymptomatic	Positive	Positive	-	Recovered
	20	Female	unknown	Household contact of outbreak 2 (Sister)	Asymptomatic	Positive	Positive	-	Recovered
	15	Female	unimmunized	Direct contact of outbreak 2 (neighbour)	Asymptomatic	Positive	Positive	-	Recovered
	20	Female	unknown	Direct contact of outbreak 2 (neighbour)	Asymptomatic	Positive	Positive	-	Recovered
	11	Female	Partially immunized	Household contact of outbreak 2 (neighbour)	Asymptomatic	Positive	Positive	-	Recovered
3 Haahorial village, Lahoal, Dibrugarh	18	Male	Claimed to be immunized (No document)	Index case 3	Pain in throat for 3 days (membrane seen)	Positive	Positive	-	Recovered

[Table/Figure 4]: Outbreak line list of diphtheria cases.



[Table/Fig-5]: Expression of *C. diphtheriae rpoB* gene (105 bp) and *tox* gene (226 bp) by Multiplex PCR in Lane 4-7 (positive samples); 50 bp ladder in Lane 1; In house positive control in Lane 2; negative control in Lane 3.

relatively mild clinical course, high case fatality rates have been reported even in recent outbreaks [3,4]. The carriers are also important source of infection, the ratio being 95 carriers for 5 clinical cases [6].

In our study, *C. diphtheriae* was isolated from ten (10) out of thirty three (33) cases with overall mortality of 20% (2/10). Three were index cases and rest of them were laboratory confirmed asymptomatic close contacts. Multiplex PCR result showed expression of both *C. diphtheriae* specific *rpoB* gene and *tox* gene by all the culture isolates [Table/Fig-5]. Follow up culture of throat swab for two occasions from all cases were found negative.

In our study, most of the cases were either unimmunized or with unknown immunization status. The unusual feature of the outbreaks was that all the cases were older than 10 years. A few previous reports from various parts of the country have revealed an increased incidence of diphtheria cases in adults [7-9]. Nandi et al., from Assam reported 59% of cases of >5 years of age group [10]. Saikia *et al.,* also described the shift in age of diphtheria cases (100% cases were >5 years of age) during the last reported outbreak in Dibrugarh district, Assam [2]. Serological studies in many countries have revealed that due to lack of adult vaccination and natural immunity a high proportion of adults become susceptible to diphtheria [11]. The potential for outbreaks of diphtheria in the community may be enhanced when there are susceptible adults and unimmunized children in the same community [12].

The occurrence of repeated diphtheria outbreaks in Dibrugarh district involving adolescents and adults reflects poor immunization coverage in previous decades. The adult vaccination in India has not been implemented yet although WHO recommends Td combination vaccination for unvaccinated individuals of 7 years of age and older [13]. The use of multiplex PCR is becoming increasingly important in the diagnosis of infectious diseases and could be a simple and fast alternative procedure for identification of *C. diphtheriae*, including screening for toxigenic and non-toxigenic strains [14]. In the present study, all the *C. diphtheriae* isolates were found to be toxigenic strains as their expression of tox gene was noted in multiplex PCR results.

LIMITATION

Inability to measure the serum antibody titre against diphtheria toxin and to perform phylogenetic analysis of the *C. diphtheriae* strains are the limitations of our study which necessitates further research.

CONCLUSION

The present study demonstrated that toxigenic strains of *C. diphtheriae* are circulating in this geographical location which indicates the need for constant epidemiological surveillance ensuring early detection of diphtheria outbreaks and review the efficacy of immunization programme. Recent outbreaks have also highlighted the need of availability of adequate quantities of diphtheria antitoxin for quick medical management of cases.

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