

A Study of Outbreak Cases of Diphtheria Diagnosed At Civil Hospital, Ahmedabad

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Abstracts: Background & objective: Most of the vaccine- preventable diseases have shown a decline with the advent of National immunization schedule but Diphtheria is still present in some region of India. The purpose of this study is to diagnose outbreak cases of Diphtheria. Method: This study was performed during JUNE 2016 to OCTOBER 2016 at the Bacteriology Laboratory of Microbiology Department, Civil Hospital, Ahmedabad- as IDSP state reference laboratory. Two throat swabs received from each suspected patient with detailed clinical and immunization history. One swab was inoculated in selective and enrichment culture media while another was used for microscopic examination for isolation of *Corynebacterium diphtheriae*. Result: Out of total 126 samples *Corynebacterium diphtheriae* was isolated in 10 samples. Among 10 cases, 7 were dead, so case fatality rate is 70%. Out of 7 deaths, 4 patients belonged to under 5, while 3 belonged to 6-12 years of age group. Interpretation & Conclusion: The majority of cases are reported from children who were non-immunized or partially immunized against Diphtheria. Persistence or resurgence of Diphtheria is mainly due to low coverage of primary immunization as well as booster doses and inaccessible areas. Targeted immunization coverage is required specifically in inaccessible areas. [Sathwara N NJIRM 2017; 8(2):55-58]

Key Words: Case fatality rate, *Corynebacterium diphtheriae*, Immunization, Under five proportional mortality rate.

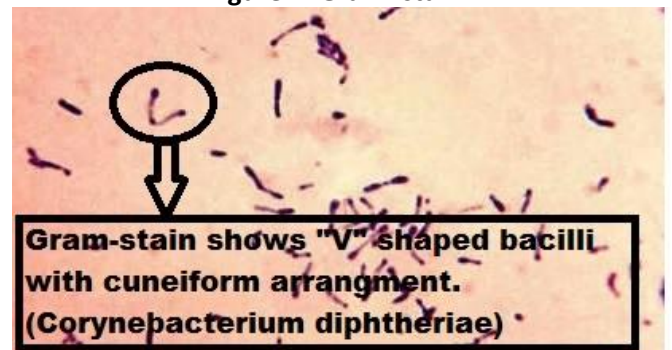
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Introduction: Diphtheria is an acute, toxin-mediated, infectious disease caused by the bacterium *Corynebacterium diphtheriae*. Despite the Universal Immunization Programme of India, which offers 3 doses of the Diphtheria, Pertussis and Tetanus (DPT) 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. In 2008, India contributed 6081(86.66%) of the 7017 diphtheria cases reported globally.¹ Ahmedabad District of Gujarat State in 2008–2009, administrative data shows coverage of 90%, while the evaluated data from Regional Resource Centre shows coverage of 78%.² As outbreak of diphtheria reflects the impact of immunization, with this view the outbreak investigation was carried out to assess the diphtheria outbreak pattern and case fatality rate of samples received at Civil Hospital Ahmedabad.

Methods: This study was performed during JUNE 2016 to OCTOBER 2016 at the Bacteriology Laboratory of Microbiology Department, Civil Hospital, Ahmedabad- as IDSP state reference laboratory. 2 throat swabs received from each suspected patient with detailed clinical and immunization history. 1 swab was inoculated in selective and enrichment culture media while another was used for microscopic examination for isolation of *Corynebacterium diphtheriae*.

Microscopic examination: The positive cases show characteristic bacilli with dark blue metachromatic granules at the ends of bacilli on Albert's stain & Gram's stain shows gram-positive bacilli arranged in Chinese letter (Figure 1).

Figure 1: Gram- stain



Selective and Enrichment culture media: Growth on TBA (tellurite blood agar) showed black colonies & on Loeffler's serum slope the colonies were small, circular white opaque, enlarged with a distinct yellow tint on continued incubation.

Biochemical reaction: It is catalase positive, ferment glucose and maltose but not sucrose. Based on cultural characteristics, stains & biochemical reactions the isolate was suspected as *Corynebacterium diphtheriae* (Table-1).

Result: Diphtheria which was common among less than five years age children in the past, is now affecting older children (5-19 years) and adults.³

Demography: In this study, 126 samples received among them *C.diphtheriae* isolates in 10 (7.93%) samples. Out of 10 positive cases, 7 were dead, so case fatality rate is 70% (Figure-2). Out of 7 deaths, 4 patients belonged to under 5, while 3 belonged to 6-12 years of age group, so under 5 proportional mortality rate is 57.14% (Figure-3). Of the 10 positive cases, 5 were males, and 5 were females (M: F ratio-1:1).

Clinical features: The most common clinical manifestation observed during the oral examination were exudative follicular tonsillitis with hypertrophy with a grayish white pseudomembrane coat and pharyngeal congestions (100%) and fever (90%). Removal of the pseudomembrane reveals bleeding, in contrast to the true membrane which can be easily separated. The typical presentation was Bull-neck appearance (70%) and cervical lymphadenopathy (60%). Other findings were breathlessness, cough, and sore throat. Among 10 positive cases, 3 patients recovered by using prompt antibiotic therapy with penicillin or erythromycin and supportive therapy.

According to the WHO –UNICEF estimates⁴, the DPT3 coverage was 66% in 2008, whereas, according to the three National Family Health Surveys^{5,6}, DPT3

coverage during 1992-2006 was only 52-55%. Immunity acquired through primary immunization wanes in early childhood. Hence adequate coverage of booster doses is equally important.⁷

Figure 2: Case fatality rate

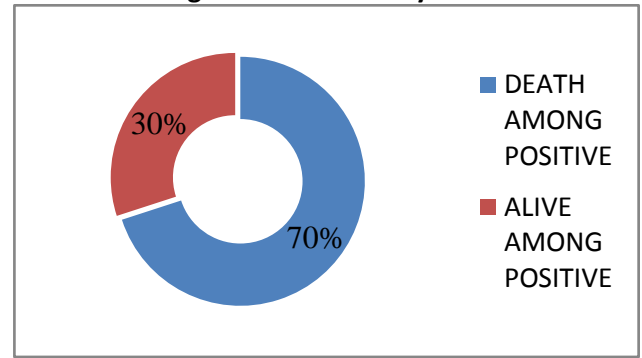


Figure 3: Under 5 proportional mortality rate

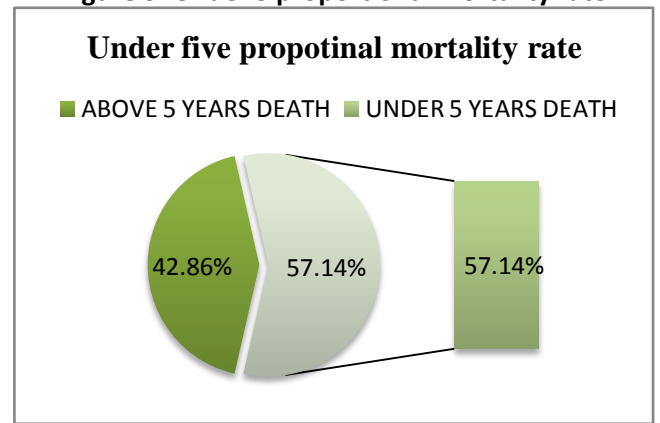


Table-1: Diagnosis based on

Location Of Cases	Age(year)/Sex	Culture for <i>C.diphtheriae</i>	Stains		Out come
			Gram Stain	Albert stain	
General Hospital Palanpur	3/Female	Positive	+	+	Death
	1/ Female	Positive	+	+	Death
	11/Female	Positive	+	-	Death
Admitted At Civil Hospital Ahmedabad From nearby areas	4/ Female	Positive	+	+	Death
	6/ Male	Positive	-	-	Recover
	12/ Male	Positive	+	-	Recover
	7/ Male	Positive	+	+	Death
	6/ Female	Positive	-	-	Recover
	8/ Male	Positive	+	+	Death
	4/ Male	Positive	-	-	Death

Discussion: *Corynebacterium diphtheriae* (*C. diphtheriae*) is a gram-positive bacillus that exists in 4 biotypes (*gravis*, *mitis*, *belfanti* and *intermedius*). Severe disease is associated with the *gravis* biotype, but any strain may produce toxin. The major virulent

factor of *C. diphtheriae* is a potent exotoxin that inhibits protein synthesis. Clinically, an illness characterized by laryngitis, pharyngitis or tonsillitis, and an adherent membrane of the tonsils, pharynx,

and/or nose plus laboratory isolation of *C. diphtheriae* by culture is required to confirm diphtheria.

For *C. diphtheriae* isolation, swab specimens should be obtained from multiple, inflamed areas of the oropharynx, nasopharynx or cutaneous lesion, and in the presence of pseudomembrane, from beneath the membrane.⁸ Other organisms including other corynebacterium species or 'diphtheroids' are considered as 'normal mouth flora'. The presence of *C. diphtheriae* in the initial throat swab specimen might have been missed.

C. diphtheriae colonies appear small, grayish, smooth and non-haemolytic on blood agar. It grows on various selective media, including cystine-tellurite, modified Tinsdale or Loeffler medium. On modified Tinsdale medium, it produces black colonies surrounded by brown haloes.⁸ Once diphtheria is suspected, toxigenicity test should be done. Traditionally, toxigenicity is determined by gel-diffusion immunoprecipitation reaction (Elek test) and other methods include enzyme immunoassays and nucleic acid amplification for detection of the regulatory gene for toxin production (*dtxR*) and diphtheria toxin gene (*tox*) but these tests were not performed.

Clinical diagnosis of diphtheria is a matter of urgency. A suspected patient should be isolated to minimize the risk of toxigenic strain spread. Close monitoring and airway management are mandatory in severe cases. Prompt antibiotic therapy with penicillin or erythromycin eradicates the organism and hastens recovery. Early administration of diphtheria antitoxin, the cornerstone of diphtheria therapy⁹ neutralizes the exotoxin before it is bound by the host cell. Once the cell internalizes the toxin, cell death is imminent. Diphtheria vaccines are based on diphtheria toxoid, a modified bacterial toxin that induces protective antitoxin. Diphtheria toxoid combined with tetanus and pertussis vaccines (DTWP) has been part of the WHO EPI since its inception in 1974. A few previous reports from various parts of the country have revealed an increased incidence of diphtheria cases in adults.¹⁰⁻¹²

Limitation: Inability to measure the serum antibody titer against diphtheria toxin and to perform a phylogenetic analysis of the *C. diphtheriae* strains is

the limitations of our study which necessitates further research.

Conclusion: Fatal diphtheria in a patient who was most likely partially immunized or non-immunized reaffirms the vital role of the EPI and the importance of efforts to ensure a high uptake of the scheduled immunizations. Clinical suspicion is important to aid diagnosis, microbiological confirmation and management of diphtheria, as the disease is rare in the era of successful immunization programs. Microbiological vigilance plays an important role in early detection of infection as well as outbreaks.

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