

Emergence of Diphtheria in Western Part of Gujarat - A Microbiological Case Series from a Tertiary Care Hospital of Rajkot

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Abstract

Objectives- The burden of diphtheria is more than what it seems to be, so early diagnosis and appropriate treatment is needed to prevent catastrophe. The study is aimed to stipulate the importance of microbiological diagnosis in cases of diphtheria. Thus, we report some confirmed cases from this region. **Materials and Methods-** 17 clinically suspected cases were studied from October 2020 to February 2021. Two throat swabs were collected. One swab was used for making two direct smears which were subjected to Gram stain and Albert stain respectively. Another swab was inoculated on Nutrient agar, Mac-conkey agar, Blood agar, Chocolate agar and Potassium tellurite blood agar (PTBA). Growths were observed after 48hrs of incubation at 37 °C aerobically. Colonies isolated from PTBA were subjected to Gram stain and Albert's stain. **Results-** 6 out of the 17 cases showed positive growth on PTBA. Macroscopically grey-black opaque colonies were obtained. Microscopic examination of the Gram-stained smears showed gram positive, pleomorphic, club shaped bacilli with L and V patterns. Albert's stain showed green colored bacilli with bluish-black polar metachromatic granules. **Conclusion-** Awareness among people, widespread universal immunization coverage, availability of modern microbiological support and rapid early diagnosis are the cardinal steps to control diphtheria resurgence.

Keywords: Diphtheria, PTBA, *Corynebacterium diphtheriae*.

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INTRODUCTION

Diphtheria is endemic in most developing countries including India. The route of transmission is through aerosol and is highly contagious for an initial 2-6 six weeks in the absence of antibiotic treatment. It is a toxin-mediated, potentially fatal, acute infectious disease of the upper respiratory system caused by *Corynebacterium diphtheriae*, which may lead to obstructive pseudo-membranes in the upper respiratory tract or damage to the myocardium and other tissues [1]. Globally, there has been a decline in cases of diphtheria due to effective childhood vaccination programs. India contributes to a substantial proportion of the global burden of diphtheria [2]. Death can occur due to cardiorespiratory failure within the first 2 weeks of infection. Early diagnosis and timely intervention help to reduce the incidence, controlling the infection in the community, and decreasing morbidity and mortality in the affected individuals [3, 4]. Appropriate antibiotics and prompt antitoxin therapy should be started as soon as diagnosed. Morbidity and mortality depend upon the patient's immunization status, age at infection, time of infection, clinical type, and time of intervention. Health conditions like cardiovascular

disease and diabetes mellitus are associated with increased risk for infection with a non-toxigenic strain of *C. diphtheriae*. Intravenous drug abusers, chronic alcoholics, and the homeless have a higher risk for contracting the disease [5]. Substandard living conditions, low socioeconomic status, immunocompromised states, and incomplete immunization are risk factors for susceptibility and transmission of infection. The main reasons for the re-emergence of diphtheria in the post-vaccine era are waning of immunity with age and improved immunization rates in children which reduces circulating wild organisms and hence the opportunities for natural boosting through subclinical infections [6]. McLeod and Anderson described three different biotypes: Gravis, Mitis, and Intermedius. *C. mitis* is responsible for mild, *C. intermedius* is an intermediate form, and *C. gravis* is a more severe form of the disease [7]. Clinical correlation with laboratory findings (both microscopy and culture) helps in the confirmation of the diagnosis. Forward communication of data helps the health authorities to spread awareness, augment immunization programs, and prevent community spread. It spreads through droplet infection or close

contact from a case or carrier. Carriers are the major source of infection, the ratio being 95 carriers for 5 clinical cases [6]. So, clinical cases are only the tip of the iceberg. Regardless of the Universal Immunization Programme (UIP) of India, which offers 3 doses of the Diphtheria, Pertussis, and Tetanus (DPT) vaccine starting at 6 weeks of age, there have been several reports of either re-emergence or persistence from several Indian states in the last 5 to 10 years [8-11].

OBJECTIVES

The burden of diphtheria is more than what it seems to be, so early diagnosis and appropriate treatment are needed to prevent catastrophe. The study is aimed to stipulate the importance of microbiological diagnosis in cases of diphtheria. Thus, we report some confirmed cases from this region.

MATERIALS AND METHODS

17 clinically suspected cases were studied from October 2020 to February 2021. Two throat swabs were collected from each patient. One swab was used for making two direct smears which were subjected to Gram stain and Albert's stain respectively. Another swab was inoculated on Nutrient agar, Mac-Conkey agar, Blood agar, Chocolate agar, and Potassium

tellurite blood agar (PTBA). Growths were observed after 48hrs of incubation at 37°C aerobically. Colonies isolated from PTBA were subjected to Gram stain and Albert's stain.

RESULTS

6 out of the 17 cases showed positive growth on Blood agar, Chocolate agar, and PTBA. Macroscopically small, circular, white, hemolytic colonies were seen on Blood agar, Chocolate agar, and grey-black opaque colonies were obtained on PTBA. The colonies on PTBA when examined under a scanner of microscope showed black colonies with the grey periphery, resembling a poached egg colony. Throat commensals are inhibited on PTBA, therefore, colonies on PTBA are preferred for further laboratory diagnostic procedures. The colonies were catalase positive, oxidase negative, non-motile, and urease negative. Microscopic examination of the Gram-stained smears showed gram-positive, pleomorphic, club-shaped bacilli with L and V patterns. Albert's stain showed green-coloured bacilli with bluish-black polar metachromatic granules.

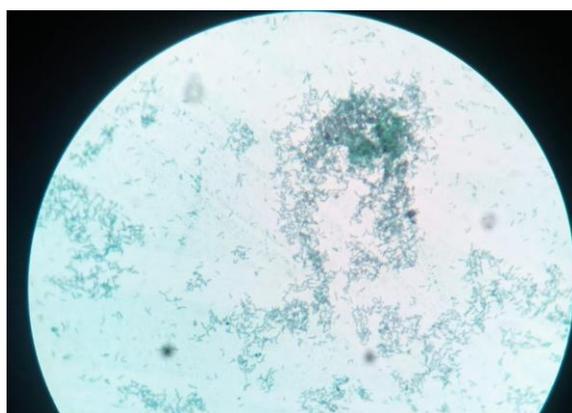


Fig-1: Albert's stain showing *C. diphtheriae* with metachromatic granules

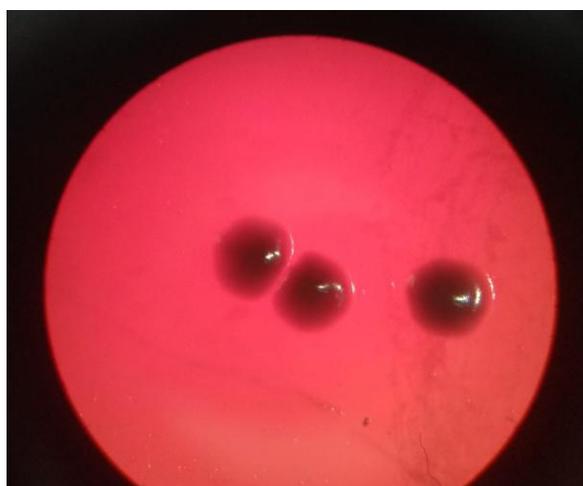


Fig-2: Colonies of *C. diphtheriae*



Fig-3: Gram stain showing *C.diphtheriae*

DISCUSSION

The number of cases reported to WHO has declined from nearly 100,000 cases in 1980 to 2,500 cases in 2015. A similar decline was also observed in India; however, India accounted for majority of diphtheria cases reported globally. During 2001–2015, nearly half of the diphtheria cases reported globally was from India. India follows the Universal Immunization Program (UIP) recommending 3 doses 4 weeks apart followed by 2 booster dose schedules. However average Diphtheria-Tetanus-Pertussis (DPT) vaccine coverage remains at only 84% [12]. The most common clinical type is nasopharyngeal diphtheria. Myocarditis is the most common complication of nasopharyngeal diphtheria. Patients who were completely immunized against diphtheria suffered from milder disease, and most of them recovered uneventfully [13]. All the 6 cases were sporadic in incidence from different geographical areas with all belonging from lower socio-economic strata and rural areas. All of them were unimmunized and presented with high-grade fever, difficulty in swallowing and breathing, neck swelling, greyish-white pseudo-membrane extending over tonsils, and pharynx, which bleeds on touch. Out of the 6 positives 4 were male and 2 were female falling in the age group 1-10years, 1 of them was below 5 years and rest of them were 5 years and above. Three patients required tracheostomy and eventually died within one week of admission. 1 of them was on oxygen therapy and later the patient died on the 8th day of admission. 2 of them who reported early to the hospital (within 1-2 days of onset of symptoms) responded well to Diphtheria antitoxin, Injection Procaine Penicillin-G and were discharged after full recovery. The clinical features of all 6 patients and colony characteristics on blood agar and PTBA, as well as morphology (on Gram stain and Albert's stain), showed resemblance to *Mitis* biotype.

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. diphtheria* [14]. In this case, although we succeeded in identifying *C. diphtheriae*, analysis for toxin production could not be performed. However, clinically

the patients were toxic and rapidly deteriorated within a span of 7 days. This can be considered as an indirect evidence of toxin-mediated disease caused by *C. diphtheriae*. The persistence of multiple foci in many regions of the country there is indeed a heightened risk of re-emergence of the disease in epidemic forms. As per CBHI data, during 2005–2014, India reported 41,672 cases (average: 4167 per year) with 897 deaths (case fatality ratio: 2.2%). Ten Indian states namely Kerala, Assam, Delhi, Gujarat, Haryana, Karnataka, Nagaland, Maharashtra, Rajasthan, and West Bengal accounted for 84% of these cases [15]. An improved understanding of the nature of each outbreak is therefore mandated to generate a pool of evidence regarding the current disease status in India. This paper aims to further existing knowledge about diphtheria in the Western part of Gujarat.

CONCLUSION

Awareness among people, widespread universal immunization coverage especially in rural areas, availability of modern microbiological support, and rapid early diagnosis are the cardinal steps to control diphtheria resurgence.

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