Cureus
Part of SPRINGER NATURE

Open Access Case Report

From Pseudomembrane to Molecular Confirmation: A Laboratory-Confirmed Outbreak of Toxigenic Respiratory Diphtheria in Nagpur, India

Review began 10/04/2026
Review ended 10/09/2026
Published 10/31/2026

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DOI: 10.7759/cureus.118897

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Abstract

Diphtheria is a potentially severe bacterial infection caused predominantly by toxin-producing *Corynebacterium diphtheriae* (*C. diphtheriae*). Although widespread immunization has substantially reduced the burden of disease, diphtheria continues to occur, particularly among individuals who are unvaccinated or incompletely vaccinated. Transmission occurs mainly through respiratory droplets and close person-to-person contact, and congregate living environments may facilitate transmission and the occurrence of clusters. Early recognition, prompt laboratory confirmation of toxigenic *C. diphtheriae*, appropriate treatment, and timely contact tracing and prophylaxis are essential to prevent further transmission.

We report four epidemiologically linked cases of laboratory-confirmed toxigenic respiratory diphtheria involving three minors and one young adult male associated with an educational residential institute. All four patients presented with fever, throat pain, odynophagia, tonsillar enlargement, and tonsillar pseudomembrane formation. Direct throat smears from all four patients demonstrated characteristic findings on Albert staining. Throat specimens yielded characteristic colonies on blood agar, chocolate agar, and potassium tellurite agar. The isolates were catalase-positive, glucose-fermenting, nitrate-reducing, and gelatinase-negative. All four isolates were confirmed as *C. diphtheriae* by a reference laboratory. Elek's test was positive, confirming toxin production, while real-time PCR detected *C. diphtheriae* and the *toxA* gene. All patients received targeted antimicrobial and supportive management. Prophylaxis was administered to all exposed contacts, following which no further cases were reported from the institute.

This cluster highlights the occurrence of toxigenic respiratory diphtheria among adolescents and young adults presenting with tonsillitis and membrane formation. Clinical suspicion, epidemiological linkage, conventional microbiology, Elek's test, and molecular confirmation enabled definitive diagnosis. Congregate settings require prompt contact investigation and vaccination-status verification following identification of a diphtheria case.

Categories: Public Health, Epidemiology/Public Health, Infectious Disease
Keywords: albert stain, corynebacterium diphtheriae, diphtheria, elek test, outbreak, tox-a

Introduction

Diphtheria is a vaccine-preventable bacterial infection caused predominantly by toxigenic strains of *Corynebacterium diphtheriae* (*C. diphtheriae*). Diphtheria toxin can cause severe systemic complications, including myocarditis and peripheral neuropathy [1]. Despite the availability of effective vaccines, diphtheria remains a public health concern in India, with outbreaks frequently affecting school-aged children and adolescents [2,3]. This age distribution may partly reflect declining vaccine-induced immunity over time and inadequate booster-dose coverage. A national serosurvey of Indian children aged 5-17 years found that only 29.7% had protective antibody concentrations, while 59.8% had partial immunity, indicating a substantial population susceptible to infection [2]. These findings underscore the importance of completing primary immunization and ensuring timely booster doses.

Diphtheria spreads predominantly through respiratory droplets and close contact, facilitating transmission in crowded and congregate environments [3]. Respiratory disease classically presents with an adherent pharyngeal or tonsillar pseudomembrane, which may bleed if forcibly removed. Delayed diagnosis and treatment increase the risk of serious complications and death, emphasizing the need for prompt clinical recognition and appropriate management [1,3].

Laboratory diagnosis involves the isolation and identification of *C. diphtheriae*, followed by assessment of toxin production. The Elek test demonstrates toxin expression, whereas molecular detection of the *tox* gene provides complementary evidence of toxigenic potential; detection of the gene alone does not confirm active

toxin production [4,5]. Identifying epidemiologically linked cases is equally important because early notification, contact tracing, assessment of vaccination status, and appropriate prophylaxis can help prevent further transmission.

We report four epidemiologically linked cases of toxigenic respiratory diphtheria involving adolescents and a young adult in Nagpur, Maharashtra, India. This case series highlights their clinical presentations, conventional and molecular microbiological findings, and the importance of recognizing clusters promptly to facilitate laboratory confirmation and coordinated outbreak-control measures.

Case Presentation

A total of four patients were included in the study. The first two siblings presented to the ENT OPD at a tertiary care hospital in Nagpur, Maharashtra, India, and were residing at the same residential educational institute for approximately three months. Both patients were from Assam, a state in the northeastern region of India, with unknown immunization histories. They declined admission and returned to their hometown after examination and throat-swab collection. The other two patients in our study presented similar clinical features and were subsequently identified from the same epidemiological network. The patients had completed their immunization schedules up to five years of age. Field investigation identified around 160 residential students residing at the institute. Other students remained asymptomatic. The occurrence of membranous tonsillitis in epidemiologically linked individuals prompted investigation for respiratory diphtheria.

Case 1

A 21-year-old young male adult presented with fever and throat pain on swallowing for five days. There was no history of trauma or breathing difficulty. Oropharyngeal examination revealed grade II tonsillar hypertrophy, a yellowish-white pseudomembrane covering both tonsils, with congestion of the posterior pharyngeal wall (Figure *1a*). The membrane bled on attempted removal. There was no bull neck appearance or palpable lymphadenopathy. No clinical evidence of myocarditis or neurological involvement was observed. General and systemic examinations were otherwise unremarkable.

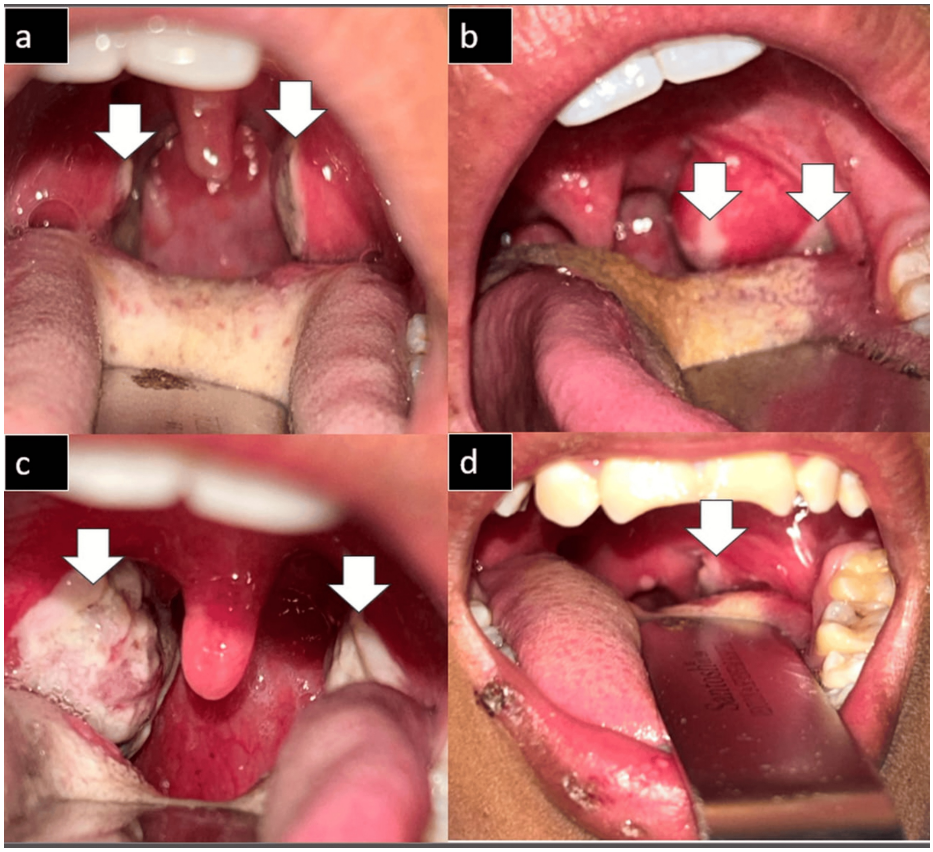


FIGURE 1: (a-d) Clinical presentation of diphtheria cases present in our study

Clinical presentation showing oropharyngeal involvement with tonsillar enlargement, adherent whitish-grey pseudomembrane, and pharyngeal congestion in all four cases respectively.

Case 2

A 15-year-old male student, the younger sibling of Case 1, presented with throat irritation, odynophagia, fever, and cold symptoms for three days. Examination revealed bilateral Grade II tonsillar enlargement with whitish membranes over the left tonsils and posterior pharyngeal congestion (Figure *1b*). Bleeding occurred when attempts were made to remove the pseudomembrane. Neck swelling and palpable lymph nodes were absent. Clinical assessment revealed no signs suggestive of cardiac or neurological complications, and the remaining general and systemic examination findings were within normal limits.

Case 3

A 15-year-old male student presented with acute tonsillitis and was admitted for evaluation. Examination revealed bilateral Grade II tonsillar enlargement, a membrane over both tonsils, and posterior pharyngeal congestion (Figure *1c*). The pseudomembrane showed bleeding upon attempted removal. No cervical swelling or palpable lymphadenopathy was detected. There were no clinical features suggestive of myocarditis or neurological complications, and the general and systemic examinations revealed no additional abnormalities. In view of the clinical findings and epidemiological link, diphtheria was suspected.

Case 4

A 13-year-old male student presented with acute tonsillitis, characterized by bilateral Grade II tonsillar enlargement, whitish membrane formation over the left tonsil, congestion of the posterior pharyngeal wall, and blister lesions over the lips (Figure *1d*). Attempts to dislodge the tonsillar pseudomembrane resulted in bleeding. The patient had no bull neck appearance or clinically detectable lymph node enlargement. Cardiovascular and neurological assessments revealed no evidence of diphtheria-related complications, while the remaining systemic examination was unremarkable. The presence of these findings, together with the epidemiological association with the other cases, prompted suspicion of respiratory diphtheria.

Microbiological workup

Throat swabs were collected from all four patients, targeting the tonsillar lesions. Direct smears were examined by Gram and Albert staining. Gram staining showed non-spore-forming, slender Gram-positive bacilli with club-shaped ends and cuneiform arrangement. Albert staining demonstrated uniformly stained bacilli with metachromatic granules, predominantly at the poles. The findings were suggestive of *Corynebacterium* species. Specimens were cultured on blood agar, chocolate agar, and potassium tellurite agar. Whitish-grey, circular colonies were observed on blood and chocolate agar, while black pinpoint colonies developed on potassium tellurite agar, suggestive of *C. diphtheriae* (Figure *2a*-*2d*). The isolates were non-motile, catalase-positive, glucose-fermenting, nitrate reduction-positive, and gelatinase-negative. All three isolates were referred to the Department of Microbiology, Christian Medical College, Vellore, and the fourth was confirmed by the Molecular Reference Laboratory, Kasturba Hospital for Infectious Diseases, Mumbai. The reference laboratories confirmed all four isolates as *C. diphtheriae*, with positive Elek tests and *toxA* PCR. Thus, the combined culture, phenotypic, toxigenicity, and molecular findings confirmed toxigenic *C. diphtheriae* infection in all four patients.

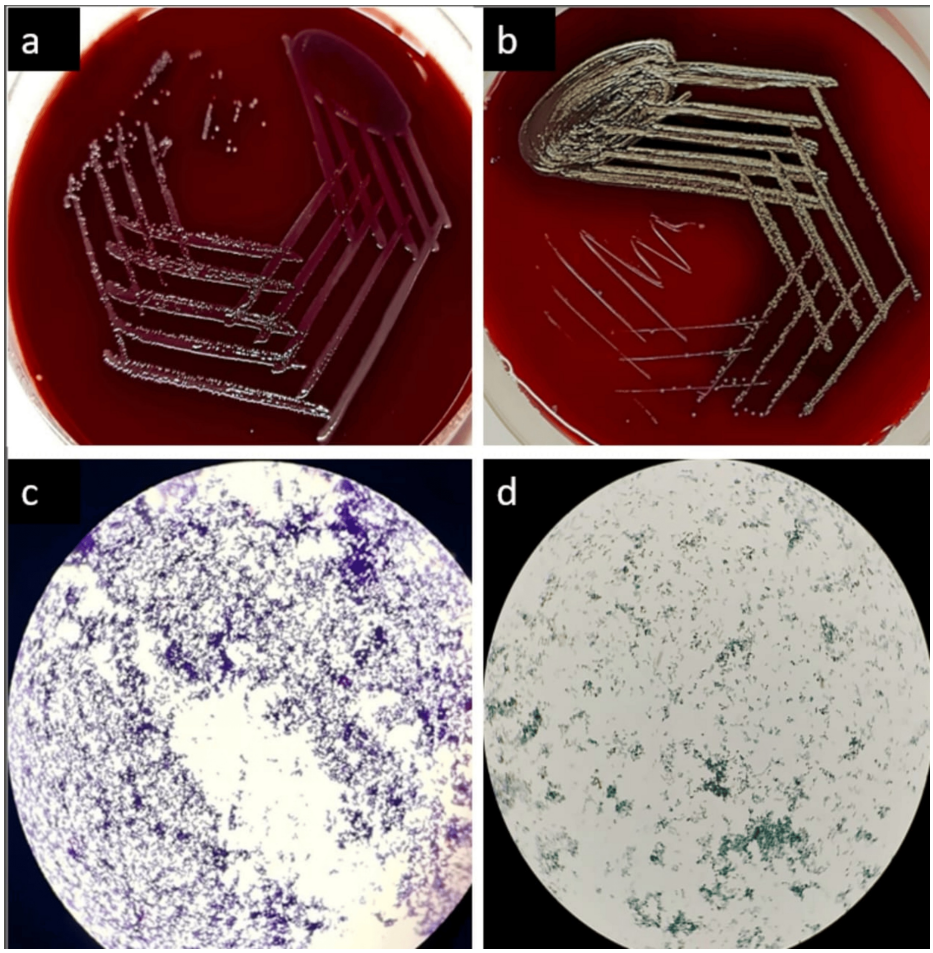


FIGURE 2: (a-d) Microbiological features of Corynebacterium diphtheriae

(a) Whitish, grey colony on blood agar; (b) Black-coloured colonies of *C. diphtheriae* on potassium tellurite agar; (c) Gram stain showing slender, non-spore-forming, Gram-positive bacilli (100x); (d) Albert stain demonstrating *C. diphtheriae* bacilli with characteristic metachromatic granules (100x).

Follow-up and treatment

The first two patients declined hospital admission and were managed in home isolation with erythromycin 500 mg for 14 days. The other two patients were admitted in isolation and treated with oral erythromycin (40-50 mg/kg/day, every 6 hours) for 14 days and diphtheria antitoxin (20,000 IU intravenously). Probable contacts received azithromycin 500 mg for three days as prophylaxis. All four patients improved following treatment. No additional symptomatic cases were identified, and a field investigation yielded no further cases from the institute.

Table *1* shows a summary of the four patients' clinical timeline.

Sr. No	Feature	Case 1	Case 2	Case 3	Case 4
1	Age/sex	21/M	15/M	15/M	13/M
2	Duration of illness	5 days	5 days	8 days	3 days
3	Symptoms	Fever, throat pain, acute tonsillitis	Fever, throat pain, acute tonsillitis	Fever, dysphagia, throat pain; acute tonsillitis; muffled voice	Fever, dysphagia, throat pain; acute tonsillitis
4	Clinical signs	Grade 2 tonsillar enlargement, bilateral, white adherent tonsillar pseudomembrane	Grade 2 tonsillar enlargement, unilateral white adherent tonsillar pseudomembrane	Grade 2 tonsillar enlargement, bilateral, white adherent tonsillar pseudomembrane	Lesion over lips, grade 2 tonsillar enlargement, unilateral, white adherent tonsillar pseudomembrane
5	Systemic Complications	None	None	None	None
6	Vaccination status	Unknown	Unknown	Fully vaccinated till 5 years of age	Fully vaccinated till 5 years of age
7	Admission status	Refused admission against medical advice	Refused admission against medical advice	Admitted on medical advice	Admitted on medical advice
8	Culture Result	*C. diphtheriae*	*C. diphtheriae*	*C. diphtheriae*	*C. diphtheriae*
9	Elek's Result	Positive	Positive	Positive	Positive
10	Real-time PCR	*C. diphtheriae* with *toxA*	*C. diphtheriae* with *toxA*	*C. diphtheriae* with *toxA*	*C. diphtheriae* with *toxA*
11	Isolation	At home	At home	Isolation ward	Isolation ward
12	Treatment	Tab erythromycin 500 mg for 14 days	Tab erythromycin 500 mg for 14 days	Tab erythromycin (40–50 mg/kg/day, every 6 hours) for 14 days, IV diphtheria antitoxin (20,000 IU)	Tab erythromycin (40–50 mg/kg/day, every 6 hours) for 14 days, IV diphtheria antitoxin (20,000 IU)
13	Outcomes	Improved	Improved	Improved	Improved

TABLE 1: Comparative clinical features of oropharyngeal lesions in four cases of Corynebacterium diphtheriae infection

Discussion

The present case series describes four epidemiologically linked, laboratory-confirmed cases of toxigenic respiratory diphtheria involving three adolescents and a young adult from the same residential educational setting. The occurrence of a cluster in a congregate environment highlights the importance of clinical suspicion, timely microbiological confirmation, notification, and coordinated public-health measures. Similar occurrences of diphtheria among older children and adolescents have been reported in Maharashtra as well as in India [6, 7].

All four patients presented with fever, throat pain, odynophagia, tonsillar enlargement, and tonsillar pseudomembrane formation. An adherent tonsillar pseudomembrane is characteristic of respiratory diphtheria, although clinical severity may vary. Diphtheria should therefore be considered in patients with membranous pharyngitis, particularly when epidemiologically linked patients present with similar features. Appropriate specimen collection and culture remain important for definitive laboratory confirmation [8].

Considering the age of patients in our study, respiratory diphtheria can occur beyond early childhood. An Indian outbreak investigation reported confirmed cases aged 4-26 years, with a median age of 12 years, and emphasized the susceptibility of older children and adolescents when vaccination or booster protection is inadequate [3]. In the present series, immunization records were unavailable for the first two patients, while the other two reportedly received vaccination only up to five years of age. These findings emphasize the importance of maintaining age-appropriate vaccination and booster coverage during later childhood and adolescence.

All four patients belonged to the same educational residential institute, which accommodated approximately 160 students. Such congregate settings facilitate close interpersonal contact and may promote respiratory transmission. The epidemiological association, followed by case notification, contact investigation, and control measures, was associated with containment of the cluster. However, molecular typing was not performed; therefore, a genetically confirmed transmission chain cannot be established. This is relevant because Indian surveillance has demonstrated considerable molecular diversity among *C. diphtheriae* isolates, with multiple sequence types identified [1].

Conventional microbiological examination provided important diagnostic evidence in this series. Microscopy offered an early presumptive clue, while culture and biochemical characterization supported identification of *C. diphtheriae*. The isolates showed catalase positivity, glucose fermentation, nitrate reduction, and gelatinase negativity. Indian surveillance has similarly emphasized integration of conventional identification with Elek testing and real-time PCR for confirmation [8,9].

Toxigenicity was confirmed by two complementary methods. All four isolates were positive by Elek's test, and real-time PCR detected the *tox*A gene. Although PCR provides rapid detection of potentially toxigenic *C. diphtheriae*, detection of the *tox* gene alone does not necessarily demonstrate toxin expression, as nontoxigenic *tox*-bearing strains have been described. Thus, the concordant Elek and molecular results provided strong evidence of toxigenic *C. diphtheriae* in these cases [10]. Duplex real-time PCR assays targeting *C. diphtheriae*-specific and toxin-associated genes can further facilitate rapid detection, while culture remains important for definitive identification and phenotypic assessment of toxigenicity.

Contact investigation and surveillance were undertaken at the residential institute. Such measures are essential because close contacts may carry *C. diphtheriae* without overt disease. An Indian report demonstrated isolation of *C. diphtheriae* among close contacts and highlighted the importance of contact tracing, microbiological evaluation, and follow-up in interrupting transmission [11].

Overall, this case series highlights that toxigenic respiratory diphtheria can affect adolescents and young adults and may occur as a localized cluster in congregate settings. Early clinical recognition, appropriate microbiological investigation, phenotypic and molecular confirmation of toxigenicity, prompt notification, and coordinated contact surveillance were central to case management and containment.

Conclusions

This case series documents four laboratory-confirmed cases of toxigenic respiratory diphtheria in epidemiologically linked adolescents and a young adult. The combination of tonsillar membrane, epidemiological linkage, positive Albert staining, characteristic culture, supportive biochemical reactions, *C. diphtheriae* PCR positivity, Elek positivity and *tox*A PCR positivity established the diagnosis in all four patients.

The cluster highlights that diphtheria should remain in the differential diagnosis of membranous tonsillitis in adolescents and young adults and that congregate residential settings can facilitate transmission. Combining conventional microbiology with Elek testing and molecular confirmation provides robust evidence of toxigenic *C. diphtheriae*. The occurrence of this cluster with incomplete or unavailable vaccination documentation further emphasizes the importance of maintaining vaccination coverage, ensuring appropriate booster protection and sustaining clinical and laboratory surveillance.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Acquisition, analysis, or interpretation of data: Smita V. Mohod, Parul S. Bansod, Nishu Sharma, Sarfaraz Ahmed, Gopal Agrawal, Dilip S. Gedam

Drafting of the manuscript: Smita V. Mohod, Parul S. Bansod, Nishu Sharma

Critical review of the manuscript for important intellectual content: Smita V. Mohod, Parul S. Bansod, Sarfaraz Ahmed, Gopal Agrawal, Dilip S. Gedam

Supervision: Smita V. Mohod, Parul S. Bansod, Nishu Sharma, Sarfaraz Ahmed, Gopal Agrawal

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

The authors gratefully acknowledge Christian Medical College (CMC), Vellore and Molecular Reference Laboratory, Kasturba Hospital for Infectious Diseases, Mumbai, for performing molecular and toxin confirmation.

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