

Case Studies

A TWO-CASE CLUSTER OF TOXIGENIC *CORYNEBACTERIUM DIPHTHERIAE* WITH MOLECULAR LINKAGE

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ABSTRACT

Diphtheria is a vaccine-preventable disease that continues to cause sporadic outbreaks in Malaysia, particularly among under-immunised populations. We report two temporally linked cases of toxigenic *Corynebacterium diphtheriae* presenting to a tertiary hospital in southern Malaysia. The first involved a 44-year-old adult male who was brought in deceased with extensive upper airway pseudomembrane formation. Two days later, a four-month-old infant presented with severe respiratory distress and required intensive care. Microbiological investigations confirmed toxigenic *C. diphtheriae* in both cases. The temporal clustering of these cases raised the possibility of an epidemiological association between them. Molecular typing using pulsed-field gel electrophoresis (PFGE) demonstrated indistinguishable strain profiles, implying circulation of a common strain and a possible shared source. These cases highlight the continued clinical relevance of diphtheria and the role of molecular methods in identifying strain linkage. Early recognition, strict infection prevention measures, and sustained vaccination coverage remain critical to preventing morbidity, mortality, and secondary transmission.

KEYWORDS: *Corynebacterium diphtheriae*, diphtheria, molecular epidemiology, infection prevention and control

INTRODUCTION

Diphtheria is a severe infection caused by toxigenic *Corynebacterium diphtheriae*. This Gram-positive bacillus has traditionally been classified into four biovars: *gravis*, *intermedius*, *mitis*, and *Belfanti*; although genomic studies no longer support this classification and instead highlight greater genetic diversity among strains. Despite the presence of established national immunisation programmes, toxigenic *C. diphtheriae* infections continue to occur sporadically in Malaysia. Although vaccination has significantly reduced the disease burden, outbreaks persist among under-immunised populations, migrants, and individuals with waning adult immunity (1). Globally, diphtheria incidence declined by more than 90% following the introduction of the Expanded Programme on Immunisation (EPI) in 1974; however, recent resurgence has been reported, with 16,911 and 22,986 cases reported worldwide in 2018 and 2019, respectively. In Malaysia, despite sustained national vaccination coverage exceeding 95%, reported diphtheria cases increased from 19 cases between 2006 and 2015 to 97 cases between 2016 and 2019, emphasising the continued risk of community outbreaks and re-emergence of the disease (2). Diphtheria remains a potentially fatal illness due to its capacity to cause acute airway obstruction, systemic toxin-mediated complications, and rapid clinical deterioration. Molecular typing techniques such as pulsed-field gel electrophoresis provide valuable epidemiological tools for identifying transmission clusters and guiding infection control interventions.

In this report, we describe two temporally associated cases of toxigenic *C. diphtheriae* presenting to a tertiary referral hospital within a short interval, which might have an epidemiological link. Molecular analysis demonstrated identical strain profiles, signifying a shared source of exposure. This case series aims to weigh the clinical and microbiological features of temporally associated diphtheria cases with identical strain profiles, emphasising the role of molecular epidemiology in identifying transmission links. It also underscores the importance of sustained public health vigilance, particularly in high-risk and under-immunised populations, and the need for early recognition and appropriate infection prevention measures in both community and healthcare settings.

Case presentation

Case 1 - Adult fatality

A 44-year-old Malay man with no known underlying medical conditions, who was detained in police custody, was brought to the Emergency and Trauma Department (ETD) in an unresponsive state. Advanced Cardiac Life Support (ACLS) was initiated; however, return of spontaneous circulation (ROSC) was not achieved, and the patient was pronounced dead. Post-mortem examination revealed extensive grey-white pseudomembranous exudates involving the oropharynx, trachea, and laryngeal tissues (Figure 1). Microbiological specimens were obtained from tracheal and laryngeal sites for further analysis (Figure 2).

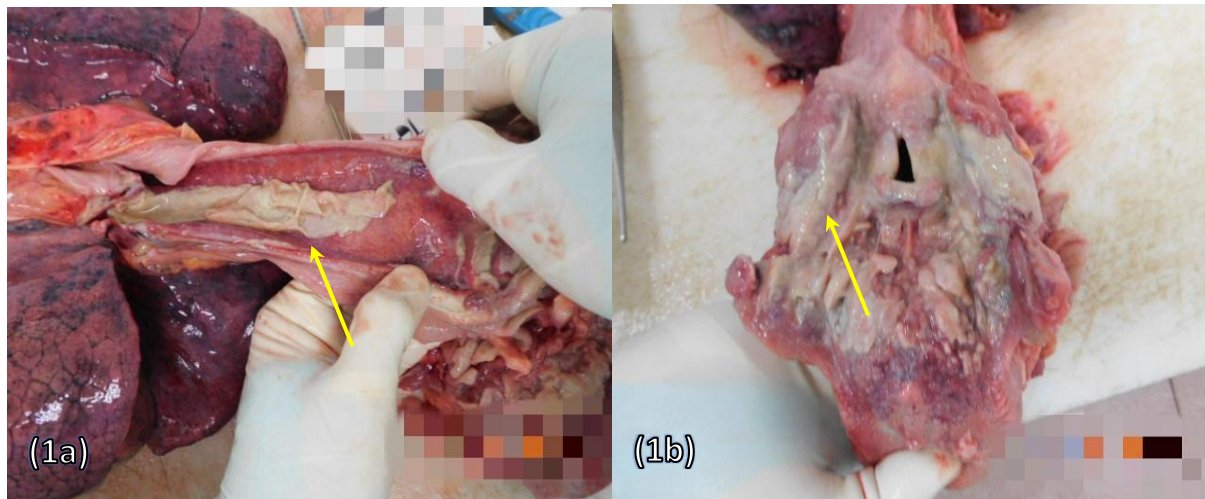


Figure 1. Forensic post-mortem of the deceased in Case 1. (a) Extension of pseudomembrane into the trachea with associated erythema of the underlying tracheal mucosa. (b) Oedematous epiglottis with whitish grey pseudomembrane covering the laryngeal mucosa. The arrows indicate the pseudomembrane.

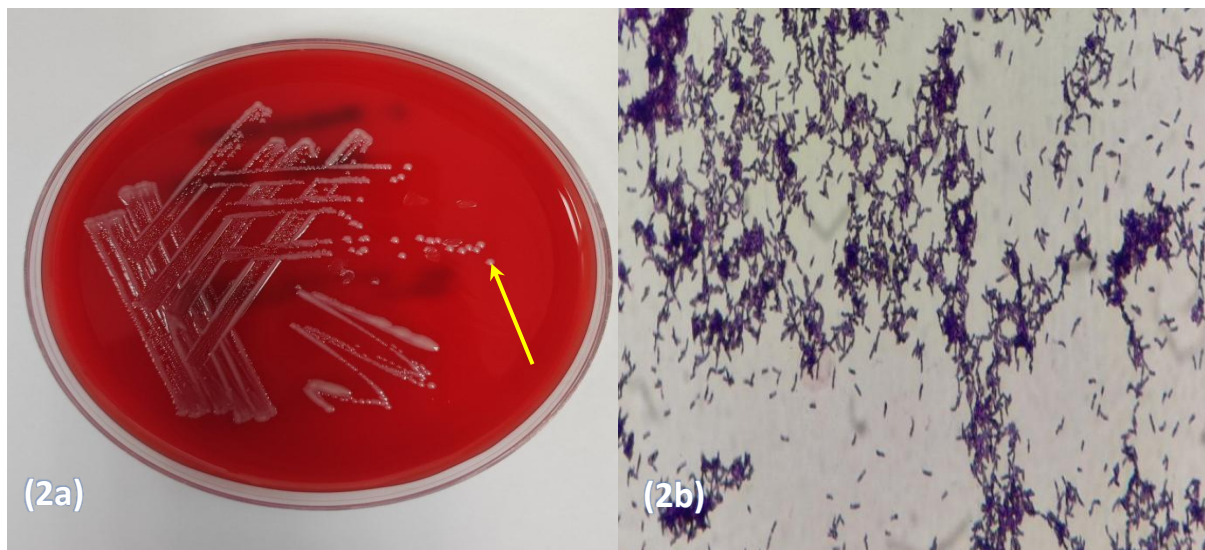


Figure 2. Microbiological findings from Case 1. (a) Subculture of the predominant colony from a laryngeal swab showing pure growth of white, opaque, non-haemolytic colonies on 5% sheep blood agar after 24 hours of incubation at 35–37°C under aerobic conditions. (b) Gram stain of the predominant colony from the subculture, visualised under oil immersion (1000× magnification), demonstrating pleomorphic Gram-positive bacilli with characteristic coryneform morphology arranged in palisades and Chinese-letter patterns.

Case 2 - Infant survivor

Two days after the presentation of the first case, a four-month-old Rohingya infant from a refugee settlement, residing with the mother in socioeconomically disadvantaged conditions and had received only birth immunisations, remained unvaccinated against diphtheria, presented to the Emergency and Trauma Department (ETD) with a three-day history of upper respiratory symptoms, poor feeding, and progressive respiratory distress.

On examination, the infant was drowsy, tachypnoeic, and poorly perfused. An intraosseous fluid bolus was administered, followed by emergency intubation due to respiratory failure. Multiple intubation attempts were complicated by peri-intubation cardiac arrest, requiring cardiopulmonary resuscitation. The infant was subsequently admitted to the Paediatric Intensive Care Unit (PICU).

Microbiological findings are shown in Figure 3. Diphtheria antitoxin and erythromycin were initiated promptly. The infant demonstrated gradual clinical improvement and was successfully extubated on day seven of admission and was subsequently discharged well with no apparent sequelae.

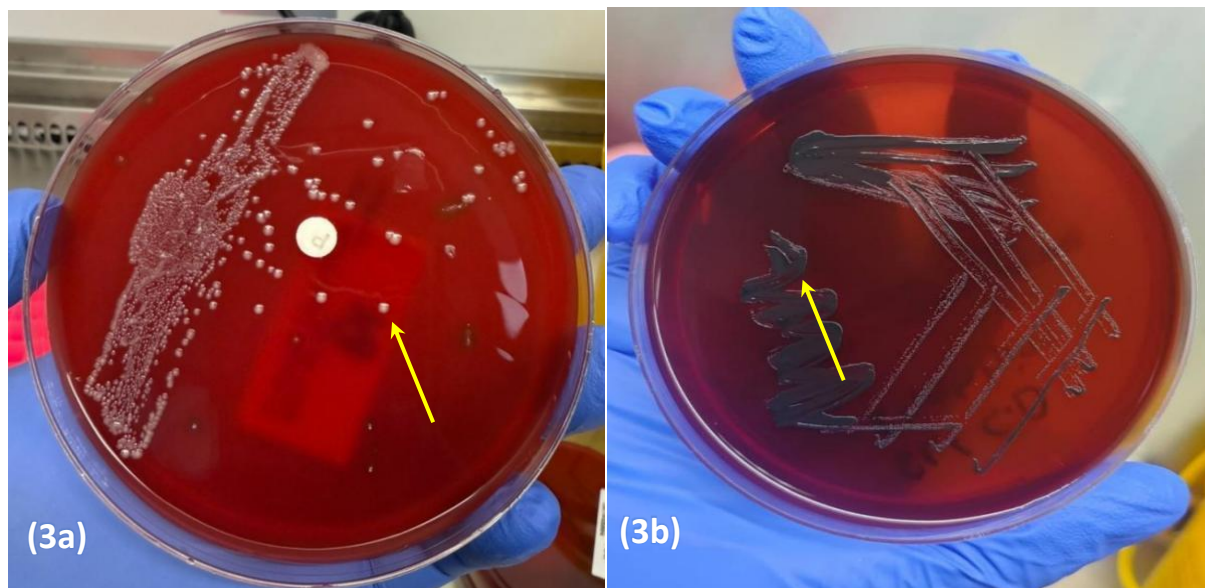


Figure 3. Microbiological findings from Case 2. (a) Blood agar (BA) plate from a tracheal aspirate culture showing mixed growth with predominant white, opaque, non-haemolytic colonies after 24 hours of incubation at 35–37°C under aerobic conditions. The arrow indicates the predominant colonies. (b) Hoyle's tellurite agar (HTA) demonstrating small greyish-black colonies after 24 hours of incubation at 35–37°C under aerobic conditions.

MATERIALS AND METHODS

Microbiological Investigation

Clinical specimens, including tracheal and laryngeal swabs (Case 1) and tracheal aspirate (Case 2), were cultured on 5% sheep blood agar and Hoyle's tellurite agar (HTA), and incubated at 35–37°C under aerobic conditions for 24–48 hours.

Bacterial identification

Isolates were identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Biotyper, Bruker Daltonics, Germany) according to the manufacturer's standard operating procedures. Briefly, a small amount of fresh bacterial colony grown on blood agar was applied onto a MALDI target plate and overlaid with 1 µL of α -cyano-4-hydroxycinnamic acid (HCCA) matrix solution. The sample was air-dried prior to analysis. Spectra were acquired using the Bruker Biotyper system and compared against the Bruker reference database. Identification scores were interpreted according to the manufacturer's criteria, where scores ≥ 2.0 indicated high-confidence species-level identification, scores between 1.7–1.99 indicated genus-level identification, and scores < 1.7 were considered unreliable. Isolates were referred to the Institute for Medical Research (IMR) for toxigenicity testing using a modified Elek test. Polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE) were performed at designated public health laboratories.

Toxigenicity testing

Molecular confirmation was performed by polymerase chain reaction (PCR) targeting the diphtheria toxin genes (*toxA* and *toxB*) using standard laboratory protocols at the Public Health Laboratory Johor Bahru, preparation and procedures involved were previously described (2). Toxigenicity of the isolates was subsequently confirmed using the modified Elek immunodiffusion assay (Figure 4), which was performed at the Institute for Medical Research, Setia Alam.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using the E-test method (bioMérieux, France). Minimum inhibitory concentrations (MICs) for penicillin, erythromycin, gentamicin, and vancomycin were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Molecular typing

Genetic relatedness between isolates was assessed using pulsed-field gel electrophoresis (PFGE) at Public Health Laboratory Johor Bahru. Banding patterns were analysed visually, and isolates were considered indistinguishable if identical patterns were observed.

RESULTS

Microbiological findings

Culture from Case 1 yielded growth of *C. diphtheriae* on blood agar, along with mixed respiratory flora. Gram staining demonstrated pleomorphic Gram-positive bacilli with characteristic coryneform morphology (Figure 2). Subculture of the predominant colony produced pure growth of white, opaque, non-haemolytic colonies on 5% sheep blood agar after 24 hours of incubation at 35–37°C under aerobic conditions.

In Case 2, tracheal aspirate cultures showed mixed growth with predominant white, opaque, non-haemolytic colonies on blood agar, and characteristic greyish-black colonies on HTA after 24 hours of incubation at 35–37°C under aerobic conditions (Figure 3). Gram staining showed similar pleomorphic Gram-positive bacilli with coryneform morphology.

The isolates demonstrated phenotypic and biochemical characteristics consistent with *C. diphtheriae*, including catalase positivity, nitrate reduction, urease negativity, and carbohydrate fermentation patterns with acid production from glucose, ribose, maltose, and glycogen, but not sucrose. These features, together with the observed colony morphology, were suggestive of *C. diphtheriae* biovar *gravis*.

Species identification was confirmed in both isolates using (MALDI-TOF MS), yielding high-confidence identification scores of 2.31 (Case 1) and 2.13 (Case 2) for *C. diphtheriae*.

Antimicrobial susceptibility result

Both isolates demonstrated similar antimicrobial susceptibility profiles. The isolates showed intermediate susceptibility to penicillin and susceptibility to erythromycin, vancomycin and gentamicin with comparable MIC values across both cases (Table 1)

Table 1. Antimicrobial susceptibility test.

Antibiotics panel	Case 1		Case 2	
	MIC	Interpretation	MIC	Interpretation
Penicillin	0.38	I	0.5	I
Erythromycin	0.016	S	0.023	S
Vancomycin	0.25	S	0.19	S
Gentamicin	0.25	S	0.25	S

Abbreviations: MIC= Minimum inhibitory concentrations, I= Intermediate, S= Susceptible. Interpretation derived from table 6 of CLSI document M45 3rd edition.

Toxigenicity

The PCR detected the presence of diphtheria genes, The modified Elek immunodiffusion testing was positive in both isolates (Figure 4).

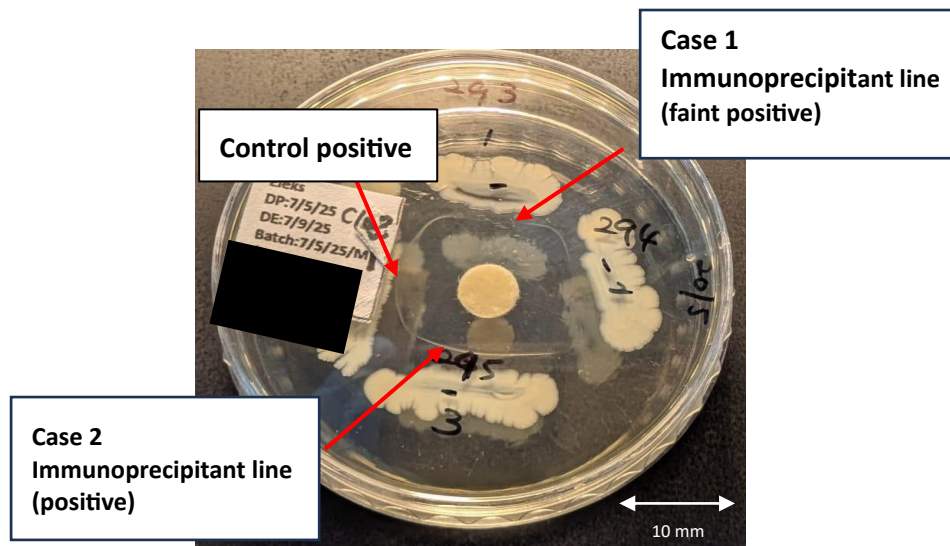


Figure 4. Positive modified Elek immunodiffusion test demonstrating toxigenic *C. diphtheriae* isolates from both cases. The isolates were cultured on Elek agar and incubated aerobically at 35–37°C for 24–48 hours. Specific precipitin lines are seen against positive controls and test cultures.

Molecular analysis and timeline

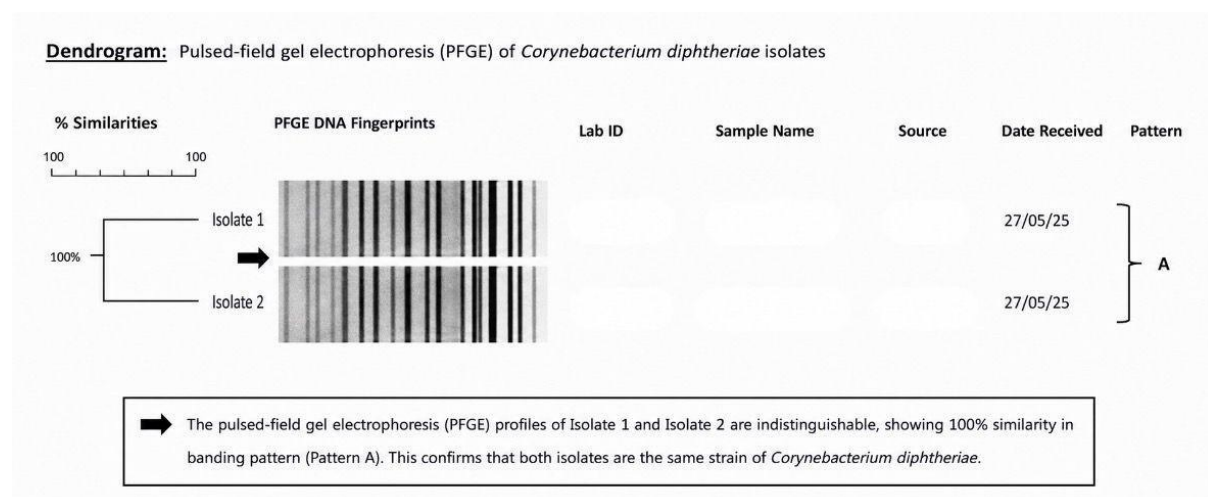


Figure 5. Pulsed-field gel electrophoresis (PFGE) analysis of *C. diphtheriae* isolates demonstrates genetically indistinguishable DNA banding patterns between Isolate 1 and Isolate 2. The dendrogram shows 100% similarity, indicating that both isolates cluster together with no detectable variation. The PFGE DNA fingerprints reveal identical banding profiles (Pattern A), confirming that the isolates belong to the same strain.

The temporal relationship between both cases is summarised in Figure 6. The occurrence of the cases within a short two-day interval, together with indistinguishable PFGE banding patterns, supports genetic relatedness between the isolates and hints circulation of a common strain within the community. However, no confirmed epidemiological connection between the two cases were identified, and direct transmission could not be established.

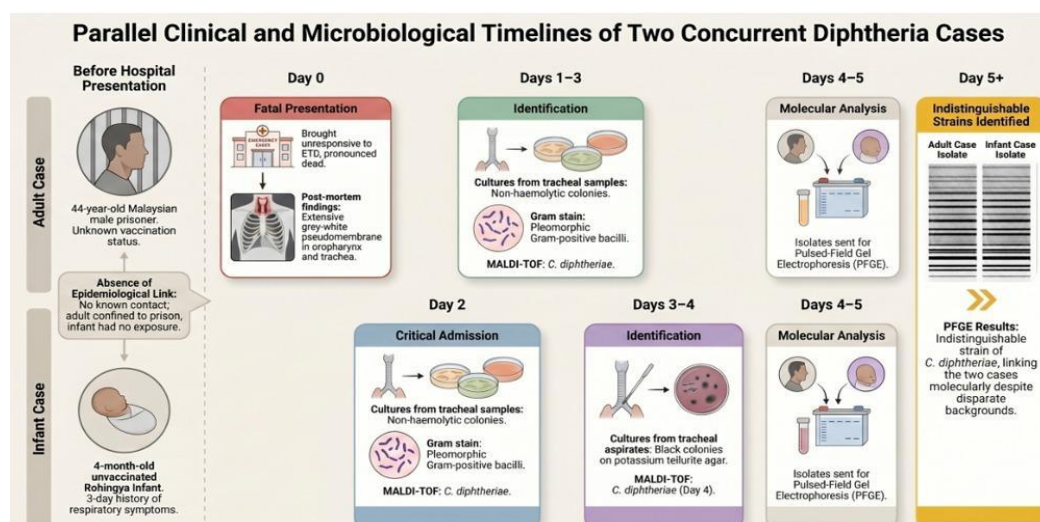


Figure 6. Timeline of clinical presentation and laboratory confirmation of two temporally associated diphtheria cases. An adult fatal case was followed by an infant case two days later. Both isolates were confirmed as toxigenic *C. diphtheriae*.

DISCUSSION

Although diphtheria is a vaccine-preventable disease, it remains a clinically significant infection in Malaysia with the potential for rapid airway compromise, myocarditis, and neurological complications. The reported increase in national case numbers following the COVID-19 pandemic marks immunisation gaps and reinforces the need for sustained public health vigilance (1, 4). Early recognition and prompt administration of diphtheria antitoxin and appropriate antimicrobial therapy remain critical determinants of patient survival and transmission control (4, 5).

Pulsed-field gel electrophoresis (PFGE) analysis demonstrated that the isolates were genetically related, hinting circulation of a common strain. However, in the absence of confirmed epidemiological data, direct transmission between the cases cannot be established. Instead, the molecular findings may reflect circulation of a common strain within the community, suggesting a possible ongoing outbreak involving a shared source or transmission network.

Although antimicrobial susceptibility testing (AST) was not essential for direct clinical management in the forensic case, it was performed as part of the microbiological characterisation of circulating isolates during the early stages of outbreak investigation. The comparable susceptibility profiles observed in both isolates provided additional supportive epidemiological evidence consistent with strain relatedness. Current WHO Interim Guidance on Laboratory Testing for Diphtheria in Outbreak Settings (2024) recommends laboratory characterisation of outbreak-associated isolates to support surveillance and public health investigations, particularly in settings where ongoing community transmission is suspected (11).

The temporal proximity of the cases, with the infant presenting two days after the adult, supports the possibility of exposure to the same circulating strain rather than direct healthcare-associated transmission. Notably, there was no known prior contact or epidemiological link identified between the two cases before hospital presentation. Although initial concern was raised regarding possible

nosocomial transmission due to overlapping emergency department exposure and involvement of common healthcare personnel during airway management and resuscitation procedures, this is less likely given the short interval between exposure and detection (3). High-acuity emergency settings nevertheless remain vulnerable to transmission events because of rapid staff turnover, multiple contact points, and time-critical interventions, which may compromise strict infection prevention practices (6). Environmental contamination was also considered but subsequently excluded.

Both cases likely originated from or were exposed to high-risk community settings. The infant resided with the mother in socioeconomically disadvantaged conditions and had incomplete immunisation status, while the adult case involved an individual from a detention setting. Both cases were associated with high-risk community settings, including a refugee settlement and a detention centre, where overcrowding and limited access to vaccination may facilitate transmission (8-10). The source of infection is therefore most plausibly linked to exposure within such high-risk community networks, although this could not be definitively confirmed. Screening of exposed individuals was limited to symptomatic contacts due to resource constraints, and asymptomatic carriers were not systematically assessed. This represents a key limitation, as asymptomatic carriage is a recognised contributor to diphtheria transmission (7-9). Consequently, potential intermediary sources may have been missing.

From a laboratory perspective, although *C. diphtheriae* was isolated on blood agar, the use of selective media such as HTA is recommended in suspected cases to enhance detection, particularly in specimens with mixed flora. According to the WHO laboratory manual for the diagnosis of diphtheria, selective media improve recovery rates and facilitate early identification. In the present series, HTA aided the isolation of characteristic greyish-black colonies in the infant case, particularly in the presence of mixed respiratory flora (12).

It is to be emphasised that this cluster was not limited to these two cases described. Public health investigations conducted by district and national epidemiology teams identified additional cases with similar molecular profiles, rendering a broader outbreak. However, detailed epidemiological linkage data were not available for inclusion in this report.

The contrasting outcomes observed in this series further illustrate the importance of early diagnosis and timely intervention. The adult case had delayed presentation and advanced disease severity resulted in a fatal outcome despite resuscitative efforts. In contrast, early administration of diphtheria antitoxin and antimicrobial therapy in the infant enabled clinical recovery. This disparity reinforces existing evidence that mortality can be substantially reduced when treatment is initiated promptly.

These cases also underscore the broader public health implications of waning adult immunity and incomplete vaccination coverage among vulnerable populations. Refugee communities with reported mortality of 0.5% to 0.8% and under-immunised groups remain at heightened risk for severe disease and onward transmission (13). Sustained booster vaccination programs, targeted outreach, and improved immunisation surveillance are therefore essential components of long-term diphtheria control strategies (14-17).

CONCLUSION

These cases highlight that diphtheria remains a serious yet preventable disease, particularly among under-immunised and high-risk populations with limited access to healthcare. The identification of genetically related strains suggests circulation of a common strain within the community and raises concern for a broader outbreak rather than confirmed healthcare-associated transmission. Strengthening vaccination coverage, including timely booster administration, alongside enhanced surveillance and early case detection, is essential to prevent further transmission. Integration of molecular epidemiology with public health investigations remains crucial for identifying transmission patterns and guiding effective control measures.

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