

Outbreak

A diphtheria outbreak in Johor Bahru, Malaysia: Public health investigation and response

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Abstract

Introduction: Diphtheria is an acute infectious disease caused by *Corynebacterium diphtheriae*. Although the incidence of diphtheria worldwide has rapidly declined following the largely successful diphtheria toxoid-based vaccines, concerns persist for those who were unvaccinated or incompletely vaccinated. In this report, we describe a recent diphtheria outbreak in Malaysia involving four confirmed diphtheria cases.

Methodology: The outbreak investigation efforts and epidemiological characteristics of a diphtheria outbreak in Malaysia are described. For all suspected cases, swabs were taken and sent for isolation of *Corynebacterium diphtheriae* and confirmation of toxigenic strains.

Results: The index case was a two-year-old child living with his family in a welfare home. Following contact tracing efforts and investigation for suspected cases, seven samples came back as culture positive for *Corynebacterium diphtheriae*. Confirmation of toxigenic strains was performed using PCR and Elek's test, which showed 100% correlation in positivity for four of the samples. All four confirmed cases were below 18 years of age, and three of them did not have complete vaccination history (two unvaccinated, one unknown). The index case eventually succumbed due to severe diphtheria with multiorgan failure while all the other cases were discharged healthy.

Conclusions: In Malaysia, despite good vaccination coverage, sporadic diphtheria outbreaks still occur. The rising trend of cases reported over the recent years underscores the need to remain vigilant. Addressing pockets of unvaccinated children and potential waning immunity levels in the population remains pivotal.

Key words: Diphtheria; disease outbreaks; *Corynebacterium diphtheriae*; vaccine-preventable diseases; Malaysia.

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Introduction

Diphtheria is an acute infectious disease, mostly caused by the gram-positive, toxin-producing strains of *Corynebacterium diphtheriae*. The diphtheria toxin consists of two subunits, each known as subunit A and subunit B, linked together by a disulfide bridge [1,2]. Subunit A carries the catalytic domain of the toxin while subunit B has the transmembrane and receptor-binding domains [3]. Diphtheria disease transmission commonly occurs via inhalation of respiratory droplets, resulting in respiratory diphtheria with the classical presentation of a pseudo membrane in the upper respiratory tract. The incubation period of *Corynebacterium diphtheriae* is usually between two to five days but may range from one to ten days [4]. Symptoms of the disease range from mild to life-threatening. In severe cases, systemic absorption of the toxin may damage other organs, usually the

myocardium and peripheral nerves, leading to potentially life-threatening complications [5].

Diphtheria had caused devastating epidemics in the past but its global burden has since eased dramatically from more than a million cases annually in the mid-1900s to 4,300 to 5,700 reported cases per year during 2006-2013 [6,7]. While the discovery and usage of diphtheria antitoxin (since 1888) and penicillin (since 1928) had contributed to better diphtheria control, the drastic reduction is mostly attributable to the diphtheria toxoid-based vaccines, particularly following the establishment and scale-up of the Expanded Programme on Immunization (EPI) in 1974 [7,8].

It is estimated that 86% of children worldwide receive the recommended 3-dose series of diphtheria-tetanus-pertussis (DTP) containing vaccines, but concerns persist for those who were not or incompletely vaccinated [7]. Diphtheria remains a significant health problem in countries with poor vaccination coverage.

The largest outbreak of the recent past occurred in eastern Europe, where more than 1,57,000 cases and 5,000 deaths were reported over a decade-long outbreak in the 1990s [9]. Outbreaks following political unrest as well as in displaced populations and infrastructure failures had also occurred over the most recent decade [8,10]. Globally, the annual number of reported diphtheria cases had been on the rise of late, with recent figures of 16,911 reported cases in 2018 and 22,986 reported cases in 2019 [11]. The recent epidemics and increasing trend of reported diphtheria cases worldwide call for renewed efforts to understand this disease better, as well as enhancing our surveillance and epidemic preparedness efforts.

In Malaysia, the DTP vaccine has had good national coverage of above 95% coverage in average since 1990, with most recent estimates of 98% and above from 2017 until 2019 [12,13]. Accordingly, this has contributed to the decrease of reported diphtheria cases nationwide from 131 cases in 1980 to below 10 cases annually in the 1990s and 2000 [11,14]. However, in recent years, diphtheria cases have surged with a total of 97 cases reported from 2016 until 2019 [11,12]. In comparison, only 19 cases were reported in the preceding ten years from 2006 until 2015. This recent increase in diphtheria cases in Malaysia calls for efforts to heighten awareness, vigilance, and surveillance of the disease.

In this report, we describe a recent diphtheria outbreak in the state of Johor, Malaysia involving a cluster of four confirmed diphtheria cases. Apart from the clinical management of the diphtheria cases, we also present the laboratory diagnostic aspects (and challenges) of the disease as well as detailing the public health response towards the outbreak, including efforts for investigation, prevention, and control.

Methodology

Johor is a state located in the southern region of Malaysia with an estimated population of 3.8 million people in 2018, with 1.6 million residing in its capital city and economic centre of the state – Johor Bahru [15]. Healthcare services in Johor are administered by the Johor State Health Department, with the Sultanah Aminah Hospital being the main referral and tertiary healthcare centre for the state. The public health division of the health department oversees the prevention and control of infectious diseases, with respective district health offices for all the ten districts in the state [16].

In February 2019, a two-year-old child accompanied by his mother was referred to the Sultanah Aminah Hospital in Johor Bahru from a nearby general

practitioner clinic for respiratory distress. The child had a history of fever and cough for two days. Physical examination findings revealed bilateral enlarged tonsils (grade 3) with large ulcers and exudates. A clinical diagnosis of acute diphtheria was made, and the Johor Bahru district health office was promptly notified. A public health team was then immediately set up and dispatched for outbreak investigation and confirmation.

Case definition and classification

Case definition for diphtheria includes clinical case definition and laboratory criteria for diagnosis [17]:

- Clinical case definition refers to an illness of the upper respiratory tract characterized by laryngitis or pharyngitis or tonsillitis and an adherent membrane (pseudo-membrane) of the tonsils, pharynx, and/or nose.
- Laboratory criteria for diphtheria diagnosis require isolation of toxigenic *Corynebacterium diphtheriae* from a clinical specimen. This detection of toxigenicity is via Elek's test or PCR.

Case classification used for case finding during outbreak investigation include [17]:

- Suspected case: Clinically compatible case without laboratory confirmation, and is not epidemiologically linked to a laboratory-confirmed case.
- Confirmed case: Clinically compatible case that is either laboratory-confirmed or epidemiologically linked to a laboratory-confirmed case.

In Malaysia, diphtheria notification is mandatory under the Prevention and Control of Infectious Disease Act 1988 [18]. All suspected cases need to be notified to the nearest district health office within 24 hours of diagnosis to be investigated [17]. Given its high infectivity and case-fatality ratio, public health response with intensive surveillance will be triggered and maintained during diphtheria outbreak situations [17,19].

Case finding

Active case detection and contact tracing were initiated by the public health team shortly after notification was received. Close contacts tracked and assessed were those who have had intimate respiratory or physical contact with the patient within the 14 days prior to the onset of sore throat [4]. This included household contacts, people with direct contact (caretakers, relatives, friends who regularly visit the patient's home) and healthcare workers exposed to the

nasopharyngeal secretions from the patient [4,19]. A case investigation form was completed for every identified case and their respective close contacts.

Case investigation and management

All suspected diphtheria cases were isolated and swabs were taken for laboratory testing. Appropriate clinical management was administered for all cases and preventive measures were taken for all identified close contacts. For both cases and contacts, diphtheria vaccination status was assessed and appropriate actions were taken for those without complete vaccination history.

Laboratory testing

Pharyngeal swabs were taken from all suspected diphtheria cases and sent for isolation of *Corynebacterium diphtheriae* and confirmation of toxigenic strains. All samples were promptly inoculated onto blood agar and Hoyles Tellurite agar upon arrival to the Johor Bahru Public Health Laboratory and incubated overnight at 37 °C. After overnight incubation, the cultures were then subjected to the standard microbiological laboratory procedures and identification using API Coryne (BioMerieux, France) [20,21].

PCR and modified Elek toxigenicity test were performed for detection and confirmation of toxigenic strains.

PCR tests were conducted at the Johor Bahru Public Health Laboratory while for the modified Elek's test, the clinical isolates were sent to the National Public Health Laboratory in Selangor.

For PCR tests, DNA extractions were carried out using QIAamp® DNA Mini Kit (Qiagen Inc, Valencia, USA). Two to five colonies were picked from the fresh cultured plates and subsequent procedures followed as described in the manufacturer's protocol. All isolates were subjected to PCR amplification of toxic gene A and B subunits. Two sets of primers (*Tox* 1: ATCCACTTTTAGTGCGAGAACCTTCGTCA and *Tox* 2: GAAACTTTTCTTCGTACCACGGGACTAA, *Dipht* 6F: ATACTTCCTGGTATCGGTAGC and *Dipht* 6R: CGAATCTTCAACAGTGTCCA) targeting the diphtheria toxin gene subunits A and B were used [22,23].

All the clinical isolates were simultaneously assayed by the modified Elek's test, which was performed by the National Public Health Laboratory in Kuala Lumpur. Preparation and procedures involved were previously described [22,24].

Data collection, data reporting, and data analysis

Information on cases and close contacts were collected and maintained – these include patients' demographic information, clinical information (date of onset, clinical signs and symptoms, hospitalization, treatment administration, and patient outcome), laboratory tests performed and results, vaccination status (and actions taken, if any), as well as relevant epidemiologic data (contact history, case classification).

Line listings containing the information above were recorded and maintained using Microsoft Office Excel 2016 spreadsheets. Using the same software, a descriptive analysis of the data collected was performed.

Ethical considerations

The study was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-21-1796-61316).

Results

A public health outbreak investigation response was initiated shortly after the notification of the index case. The index case, a two-year-old child, lived together with his mother and elder sibling in a religious welfare home that houses children from poor and underprivileged families. Upon further investigation, it was discovered that some of the children who resided in the welfare home were or may have been secondary contacts to close contacts of diphtheria cases reported in the preceding year (October to December 2018). There also appeared to be linkages among the contacts to a religious group that was associated with vaccine hesitancy sentiments.

Through active case detection and contact tracing efforts, we identified 54 contacts: 21 contacts from the welfare home (index case family members, other children, and caretakers), two contacts from the general practitioner clinic, and 31 contacts from the hospital (healthcare workers). During the period of surveillance, a total of 15 contacts were symptomatic and all of them had swabs taken for laboratory testing. From the total 16 samples (index case and 15 symptomatic contacts) sent for laboratory testing, seven samples came back as culture positive for *Corynebacterium diphtheriae*. Isolates from these seven samples were then examined further for detection and confirmation of toxigenic strains using both PCR and Elek's test. Four samples (out of seven) were positive for both A and B subunits of the diphtheria toxin gene by PCR. All four samples were also positive for Elek's test, showing 100%

correlation between conventional PCR and Elek’s test. The other three samples were negative for both toxin genes by PCR as well as Elek’s test.

Table 1 depicts the demographic and clinical characteristics for all the seven suspected cases with positive swab cultures for *Corynebacterium diphtheriae*, together with their respective laboratory testing findings. Four cases had laboratory confirmation by PCR and Elek’s test for toxigenic strains and were registered as confirmed diphtheria cases while the other three were discarded as they were non-toxigenic. All four confirmed cases were below 18 years of age, with three of them below five years old (n = 3, 75.0%). Two of the cases did not receive any diphtheria toxoid vaccination since birth (unvaccinated), one case had complete vaccination history, while the last remaining case had unknown prior vaccination history.

Clinical case management and outcome

All the seven suspected cases with positive swab cultures for *Corynebacterium* were admitted into hospital with isolation measures put in place, and prompt antibiotic therapy (penicillin or erythromycin) was administered. Apart from the index case who did not survive (described further below), all the other six patients were eventually discharged well and healthy. Upon completion of their antibiotic course, two swab samples from each patient were obtained at least 24 hours apart to demonstrate the elimination of the organism. All the samples taken yielded negative swab culture results.

For the index case (No. 001 in Table 1), diphtheria anti-toxin was also given via intravenous infusion. The patient, however, succumbed on the fourth day of admission due to severe diphtheria with multiorgan failure.

Contact monitoring and management

All the remaining contacts were monitored for clinical signs and symptoms for at least ten days from the date of their last contact with a confirmed case. There were nine symptomatic contacts from whom swabs were taken and cultured but were negative for *Corynebacterium diphtheriae* – antibiotic therapy was administered. The other remaining 38 contacts remained asymptomatic and received antibiotic prophylaxis.

Preventive measures and vaccination

Health education on the nature of infection, the mode of transmission, and importance of personal hygiene were given to all cases and contacts, as well as to their family members and caregivers. An alert was issued to all nearby healthcare facilities. Disinfection activities were also carried out in the welfare home.

Vaccination status was assessed for all cases and contacts. Those who did not have complete vaccination history were counseled and given the appropriate diphtheria toxoid dose(s) to complete their vaccination series. All cases received their catch-up diphtheria toxoid immunization during convalescence (ideally start or administer before discharge). All contacts were

Table 1. Characteristics, diagnostic tests results, and management for all suspected cases with *C. diphtheriae* isolated.

No	Age (years)	Sex	Vaccination status	Contact history	Symptoms	Signs	Isolate from swab culture	PCR test Toxin A	PCR test Toxin B	Elek's test	Outcome
001	2	M	Not vaccinated	Index case	Cough, fever, poor oral intake, rapid breathing	Enlarged tonsils with exudates and ulcer	<i>C. diphtheriae</i> isolated	Pos	Pos	Pos	Expired on fourth day (of admission)
002	4	F	Not vaccinated	Elder sibling of index case	Cough	Injected throat, cervical lymphadenopathy	<i>C. diphtheriae</i> isolated	Pos	Pos	Pos	Discharged healthy
003	4	F	Full vaccination	Contact at welfare home	Cough	Injected throat	<i>C. diphtheriae</i> isolated	Neg	Neg	Neg	Discharged healthy
004	4	M	Partial vaccination	Contact at welfare home	Cough	Cervical lymphadenopathy	<i>C. diphtheriae</i> isolated	Neg	Neg	Neg	Discharged healthy
005	4	F	Partial vaccination	Contact at welfare home	Cough, runny nose	Inflamed tonsils, cervical lymphadenopathy	<i>C. diphtheriae</i> isolated	Neg	Neg	Neg	Discharged healthy
006	4	M	Full vaccination	Contact at welfare home	Fever	Inflamed and enlarged tonsils, cervical lymphadenopathy	<i>C. diphtheriae</i> isolated	Pos	Pos	Pos	Discharged healthy
007	15	F	Unknown	Contact at welfare home	Asymptomatic	Enlarged tonsils with exudates	<i>C. diphtheriae</i> isolated	Pos	Pos	Pos	Discharged healthy

F: female; M: male; PCR: polymerase chain reaction; Neg: negative; Pos: positive.

also given appropriate diphtheria toxoid dose(s) to complete their vaccination series [4,25].

Surveillance, investigation, and response in outbreak settings

Notification of the index case in this outbreak triggered a public health response from the Johor Bahru district health office. A public health investigation was conducted to confirm and describe the outbreak, identify the source(s) or contributing factor(s), recommend and implement control and prevention measures, and communicate findings. Accordingly, a public health team of relevant healthcare professionals was assembled and a diphtheria outbreak operation room was set up in the district health office. The intensive surveillance, investigation and response efforts were maintained until two incubation periods (20 days) after the date of onset of the last confirmed diphtheria case.

Discussion

In Malaysia, despite good vaccination coverage over the past three decades, diphtheria outbreaks still occur – most of them, however, were sporadic in origin and involved small number of cases. In this report, we described a recent diphtheria outbreak involving four confirmed cases and highlighted the public health efforts in the outbreak investigation, as well as the prevention and control measures taken. There was no identifiable source of infection for the index or primary case in this cluster. While uncommon, it is possible that transmission of the causative organism may have originated from healthy asymptomatic carriers, as chronic carriers may shed the infectious organism for up to six months or more [4].

Although the diphtheria toxoid vaccine does not prevent colonization, it reduces transmission by 60%, likely via reduced symptomatic shedding of the virulent bacteria [8]. Full vaccination (≥ 3 doses) with the DTP series also had been reported to be 87% effective against symptomatic disease and 81% effective in preventing severe disease [8]. Considering its benefits, every country should seek to achieve timely vaccination with a complete primary series plus booster doses [7]. In the cluster reported, two confirmed cases were unvaccinated while one case had unknown prior vaccination history. The index case, who was unvaccinated, eventually developed severe diphtheria with multi-organ failure and did not survive. The number of confirmed cases, however, was too small to make reliable comparisons on vaccination status.

Most of the diphtheria cases reported in the recent years in Malaysia affected children, but there were also sporadic cases involving adults [19,26]. The occurrence of diphtheria among adults, as well as the recent rise of diphtheria cases, suggest that there could be waning immunity levels in the population. With this in mind, a recent study was conducted to investigate the seroprevalence of diphtheria toxoid IgG antibodies in the Malaysian population. Findings from the study revealed that about 57% of the Malaysian population have inadequate immunity against diphtheria infection, with children at age five to six years old particularly vulnerable [14]. Recommendations by the study to bring forward the booster dose to four to five years of age instead of age seven, as well as a potential booster dose for high-risk adults, merit consideration. In the outbreak reported in this study, all children (cases and contacts) were given catch-up DTP doses when necessary to complete their vaccination series, but the adults were not as there were no recommendations to provide diphtheria vaccine booster doses for adults in Malaysia [14,19].

Laboratory diagnostic methods for rapid microbiological confirmation of a clinical diagnosis of diphtheria are crucial so that timely intervention with specific treatment can be administered. There are several diagnostic methods to detect toxigenic *Corynebacterium diphtheriae* but not all are available in Malaysia [20,22]. In this report, we demonstrate the usage of two tests: PCR as a rapid and reliable tool for the detection of toxigenic strains and the modified Elek's test that detects toxin expression. The Elek's test, while confirmatory, presents challenges as they are only carried out in selected national or reference laboratories [22,27]. Additionally, Elek's test procedures are often time consuming, may be prone to misinterpretation, and sometimes need to be repeated due to contamination or inconclusive results [22].

In comparison, the PCR test for the detection of toxigenic strains is faster (approximately four hours) and the interpretation of results is simple [20,22]. Although PCR is not available in all hospitals, most major hospitals including state hospitals in Malaysia are able to perform it and capacity building to increase its availability in more hospitals may be easier than Elek's test. In a previous local study that compared the PCR test with Elek's test, there was 100% concordance between the results of both tests in the 48 strains of *Corynebacterium diphtheriae* examined [22]. Our findings in this report also showed 100% correlation between PCR test and Elek's test in the seven isolates examined, further adding to the evidence that supports

PCR as a reliable diagnostic tool to detect toxigenic strains.

At present, however, PCR test results are still not accepted as a criterion for laboratory confirmation [4, 20]. In rare cases, the presence of toxin genes in *Corynebacterium diphtheriae* isolates does not necessarily express a biologically active protein [22,28]. PCR test also should not replace bacterial culture as in some situations (poor specimen quality, delayed testing, specimens taken after antibiotics administration), PCR may be positive while culture is negative [12]. In Malaysia, isolation from bacterial culture and Elek's test are still required to fulfill the laboratory criteria for diphtheria case confirmation [17,27]. However, PCR results are still useful as they are used to guide case management decisions [27].

Appropriate and timely clinical case management of diphtheria cases is crucial to preventing life-threatening systemic complications. While antibiotics therapy and isolation may help to interrupt transmission, the mainstay definitive treatment for diphtheria cases is still diphtheria antitoxin (DAT) [8,12,25]. Administration of DAT may potentially reduce mortality by 76% (relative risk 0.24, 95% credible interval: 0.22-0.28) [8]. In the recent diphtheria outbreak in Bangladesh, 709 patients were treated with DAT with excellent outcomes (mortality < 1%) and while adverse reactions occurred in one-quarter of the patients, they were mostly mild and resolved quickly [29].

Prompt administration of DAT is critical as the disease course and outcome depend on how early from disease onset that DAT is started, with risk for complications and mortality rising by each day of DAT administration delay [4,8]. Therefore, if diphtheria is strongly suspected based on clinical diagnosis, DAT should be administered without waiting for laboratory results [4,25]. Nevertheless, DAT is not commonly available and is not often stocked by hospitals or healthcare facilities. Global stockpiles of DAT had also dwindled in the recent years due to discontinued production and expiration, as a result of reduced demand [8,30]. It is therefore important, in our preparedness for future outbreaks, to plan and coordinate the logistics of DAT distribution to prevent delays in administration.

Overall, we have presented and discussed salient points in the public health response towards a diphtheria outbreak in this report, zooming into the public health investigation efforts as well as prevention and control measures. With the rising trend of cases reported in the past few years, it is important for us to remain vigilant

and prepared for potential diphtheria outbreaks in the future. Proper case-based surveillance at various levels (from national to facility-based) must be maintained and epidemic preparedness efforts should be bolstered – this also includes strengthening laboratory capacity where necessary. Finally, we should continue to educate the public on the risks of vaccine preventable diseases as well as on the need for, and benefits of vaccination.

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Authors' Contributions

Conceptualization: PSKT, NR, HRT. Investigation and Methodology: PSKT, MJ, NFM, NSB, NR, HRT. Data curation: PSKT, MJ, NSB. Project administration: PSKT, MJ, NFM, NSB. Supervision: NR, HRT. Writing – original draft: PSKT, MJ. Writing – review & editing: NFM, NSB, NR, HRT.

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