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Diphtheria in Metro Manila, the Philippines 2006–2017: A Clinical, Molecular, and Spatial Characterization

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Background. Diphtheria is a vaccine-preventable disease that persists as a global health problem. An understanding of the pattern of disease is lacking in low- and middle-income countries such as the Philippines.

Methods. We conducted a retrospective review of the clinical, microbiological, and epidemiological features of patients admitted with a clinical diagnosis of diphtheria to an infectious disease referral hospital in Metro Manila, the Philippines, between 2006 and 2017. Cases were mapped and the distribution was compared with population density. *Corynebacterium diphtheriae* isolates from between 2015 and 2017 were examined by multilocus sequence typing (MLST).

Results. We studied 267 patients (range:12–54 cases/year) admitted between 2006 and 2017. The case fatality rate (CFR) was 43.8% (95% confidence interval, 37.8–50.0%). A higher number of cases and CFR was observed among children <10 years. Mortality was associated with a delayed admission to hospital and a lack of diphtheria antitoxin. Between 2015 and 2017 there were 42 laboratory-confirmed cases. We identified 6 multilocus sequence types (STs). ST-302 was the most common (17/34, 48.6%), followed by ST67 (7/34, 20%) and ST458 (5/34, 14%). Case mapping showed a wide distribution of diphtheria patients in Metro Manila. Higher case numbers were found in densely populated areas but with no apparent clustering of ST types.

Conclusions. Our analysis indicates that diphtheria remains endemic in Metro Manila and that the infection is frequently fatal in young children. Improved vaccine coverage and a sustainable supply of diphtheria antitoxin should be prioritized.

Keywords. diphtheria; the Philippines; diphtheria antitoxin; MLST; vaccination.

Diphtheria is vaccine-preventable disease that remains a global health problem. Toxigenic *Corynebacterium diphtheriae* can cause an upper respiratory tract infection complicated by cardiac, neurological, and renal manifestations associated with a high mortality. Transmission occurs by person-to-person route through close physical contact and respiratory droplet spread [1, 2]. Diphtheria incidence has steadily declined with the introduction of the Expanded Program on Immunization (EPI) and socioeconomic improvements [1]. Despite these efforts, diphtheria remains endemic in areas with low vaccine coverage, poor hygiene conditions, and overcrowding [3, 4]. Recent outbreaks have been reported in Indonesia, Yemen, and Myanmar/Bangladesh [4–6].

An EPI program was established in the Philippines in 1976 with a primary vaccine course of 3 diphtheria, pertussis and tetanus vaccine (DPT) doses given at 6, 10, and 14 weeks of age [7]. In 2013, a school-based immunization program was launched for children aged 6–7 years and 11–12 years [7, 8]. The World Health Organization (WHO) estimated the immunization coverage for the primary series of diphtheria vaccination to be 86% in 2016. To our knowledge, the vaccine coverage for the booster doses in the Philippines has not officially been reported [9]. Despite these efforts, diphtheria cases continue to be reported [10]. According to WHO statistics, there were an average of 56 (range, 0–188) annual cases reported between 2006 and 2017. The Philippines Department of Health (DOH) reported 104 to 168 annual cases between 2014 and 2017 [10–12].

We conducted an observational study in the National Infectious Disease hospital (San Lazaro Hospital [SLH]) in Metro Manila to describe the clinical, microbiological, and epidemiological characteristics of patients attending the hospital with clinically diagnosed and laboratory-confirmed diphtheria. We also aimed to understand the distributions of diphtheria cases by molecular types in Metro Manila.

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METHODS

We conducted the study at SLH, a 500-bed tertiary hospital specializing in infectious diseases in Metro Manila [11]. San Lazaro Hospital is the national referral center treating the largest number of diphtheria cases in the Philippines.

We analyzed patients admitted to SLH with a discharge diagnosis of diphtheria (International Classification of Diseases, 10th revision, code A36) over an 11.1-year period between 1 January 2006 to 9 February 2017. The clinical diagnosis of diphtheria was based on assessments by 2 attending physicians and defined as a respiratory illness consisting of pharyngitis, tonsillitis, or laryngitis and an adherent tonsillar or nasopharyngeal pseudomembrane. Demographic, epidemiological, and clinical data including the date of admission, age, sex, home location of the patient, and administration of diphtheria antitoxin (DAT) were extracted from the medical records. DAT was not available until March 2016 when the WHO and the DOH started to provide DAT.

In December 2015, the hospital established laboratory methods for *C. diphtheriae* isolation and confirmation and started prospective data collection using structured questionnaire sheets for all patients with clinically suspected diphtheria. We analyzed these laboratory-confirmed cases separately. The immunization history was obtained by the questionnaire, and we requested family members to present the immunization cards.

Laboratory-confirmed cases were defined as a patient with a positive result of culture for *C. diphtheriae* and/or a tox-gene polymerase chain reaction (PCR)–positive result from throat or nasopharynx swab samples.

Throat or nasopharynx swab specimens were taken at the time of admission from all patients with clinically suspected diphtheria. Brain heart infusion (BHI) broth was used as transport media. The swabs were inoculated to 5% sheep blood agar and selective Hoyle`s agar with potassium tellurite (Oxoid). Black suspect colonies on Hoyle's media that were gram-positive bacilli were identified using MALDI Biotyper (Bruker Daltonics). The Minimum Inhibitory Concentrations (MICs) of penicillin G and erythromycin were determined by E-test strips according to manufacturer's instructions (Biomerieux). Isolates were stored in glycerol broth at −80°C for subsequent tox-gene PCR.

We assessed the toxigenicity of the identified isolates by a PCR that detects the tox gene using a published method [13]. Direct toxin gene detection was also performed on the throat swabs. DNA was extracted from the 100-μL aliquot of the BHI transport broth using a QIAamp DNA Blood Mini Kit following the manufacturer's instructions (Qiagen). DNA extracted from isolates was stored at −20°C using a DNA extraction kit (Wizard Genomic DNA Purification Kit; Promega) and transported to Nagasaki, Japan for multilocus sequence typing (MLST) as described by Bolt et al [14]. Sequence types (STs) were assigned by the PubMLST database and added to this database (https://pubmlst.org/cdiphtheriae/). We generated a phylogenetic tree by using Molecular Evolutionary Genetics Analysis (MEGA) software version MEGA 7.0.26 (https://www.megasoftware.net/). A maximum likelihood tree was constructed using the concatenated sequences (2544 nucleotides) of the 7 loci used in MLST. In the analysis, the publicly available sequence of 56/S (*C. diphtheriae* isolated in Poland, 1950) was added to the alignment as an outlier. We defined a clonal complex as a cluster of isolates sharing 6 out of 7 alleles. Clonal analysis was performed using eBURSTv3 (http://eburst.mlst.net/default.asp). We generated an eBURST diagram of *C. diphtheriae* to show relatedness between isolates in this study and 703 isolates previously registered in PubMLST (https://pubmlst.org/cdiphtheriae/).

We converted the home location of the patients with diphtheria in Metro Manila into Global Positioning System (GPS) locations and conducted analysis using Geographic Information System (GIS) software (ArcGIS version 10.5; ESRI). Population data and basic maps were obtained from the PhilGIS (http://www.philgis.org/gis-data), the Philippines Statistics Authority (https://psa.gov.ph/), and Esri (http://www.esri.com/software/arcgis/arcgisonline/features/maps). We created a case map using GPS locations of clinically diagnosed diphtheria cases between January 2006 and December 2015 and culture-positive diphtheria cases by the STs between December 2015 and February 2017. We created a population-density map in Metro Manila and compared the distributions of the diphtheria cases with the population density. Kernel density analysis was used to determine hotspot areas of clinically diagnosed diphtheria cases between January 2006 and December 2015. We compared the distributions of culture-positive diphtheria by STs with the hotspot area created with the previously clinically diagnosed diphtheria cases.

We used Microsoft Access 2010 (Microsoft Corporation) for the data entry. Each case was given a study-specific identifying number. Statistical analyses were performed using Stata software (version 13; StataCorp). We conducted a statistical analysis of patient characteristics using Fisher's exact test for categorical variables and *t* test for continuous variables. We calculated risk ratios (RRs) of the effect of variables on fatal outcomes using an unadjusted logistic regression model. An adjusted logistic regression model was not used in this study because of the small sample size. Age groups were categorized into 0–4, 5–9, 10–14, 15–20, and over 20 years. We report in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement.

Ethical approval was obtained from the Research and Ethical Review Board of SLH, the Philippines (reference number:2016–015-E), and the Institutional Review Board of the Institute of Tropical Medicine, Nagasaki University, Japan (170707169). Both review boards agreed that consent from the patients was not required as this study consisted of analysis of data previously collected as part of standard clinical management.

RESULTS

Between 1 January 2006 and 9 February 2017, a total of 267 patients (mean, 24 cases/year; range, 12–54 cases/year) with clinically diagnosed diphtheria were admitted to SLH (Table 1, Supplementary Figure 1). The majority of cases were children younger than 15 years old (231 cases, 86.5%). Children aged 5–9 years (103 cases, 38.6%) were most common, followed by children younger than 5 years (83 cases, 31.1%).

A total of 117 patients died in the hospital (43.8%; 95% confidence interval [CI], 37.8–50.0) (Table 1). The case fatality rate (CFR) was highest (60.2%; 48.9–70.8%) among children younger than 5 years, followed by those aged 5–9 years (44.7%; 34.9–54.8%) (Supplementary Figure 2). The lowest CFR was observed among subjects aged 15–19 years (17.4%; 5.0–38.8%). There was no significant difference in the CFR by sex. There was a significantly lower CFR among patients given DAT as part of their clinical management compared with patients not given DAT (103/216 [47.7%; 40.9–54.6%] vs 14/51 [27.5%; 15.9–41.7%]; $P < .01$).

After introducing enhanced laboratory testing on 1 December 2015, there were 68 patients with clinically diagnosed diphtheria, of whom 42 were confirmed by laboratory testing (Table 2). *Corynebacterium diphtheriae* was isolated in 35 patients, and all isolates had a positive tox-gene PCR result. A further 7 patients had a positive tox-gene PCR on the throat swab but a negative culture result. There were 33 (94.3%) isolated strains susceptible to penicillin G (MIC ≤0.12 mg/dL) and 2 (5.7%) strains intermediate according to the Clinical Laboratory Standards Institute (CLSI M45 2016) guideline. Most isolates were susceptible to erythromycin, but 1 isolate was resistant (MIC = 2 mg/dL) [15].

We observed 18 deaths among the 42 patients with laboratory-confirmed diphtheria, giving a CFR of 42.8% (95% CI, 27.7–59.0%). The CFRs among children younger than 5 years (60%; 32.3–83.7%) and those aged 5–9 years (80%; 28.4–99.5%) were higher compared with patients aged 10–14 years (25%; 5.5–57.2%) and 15–19 years (0%), although the CIs were wide due to the small sample size (Supplementary Table 2, Supplementary Figure 3). Of patients, 33.3% were living outside Metro Manila and they had significantly higher CFRs compared with patients living in Metro Manila (64.3% [35.1–87.2%] vs 32.1% [15.9–52.4%]; $P = .049$). Patients attending the hospital within 4 days after onset of symptom had a significantly lower CFR than those admitted after 5 days or more (28% [12.1–49.4%] vs 64.7% [38.3–85.8%]; $P = .03$). The RR of death among patients with a chronic disorder compared with patients without a chronic disorder was significantly increased (RR, 2.11; 95% CI, 1.16–3.86).

Twenty (47.6%) of the 42 patients with laboratory-confirmed diphtheria reported having received 3 primary doses of DPT vaccine, but only 8 were confirmed by inspection of their immunization record card. Among the fully immunized patients confirmed by their immunization card, there was 1 death of a 9-year old patient. There was no significant difference in the CFR between subjects with a history of receiving 3 doses of primary vaccinations than in those without any history of the vaccine (Table 2). None of the patients reported receiving a booster dose of diphtheria vaccine. All cases were treated with penicillin G. Among the 30 patients who received DAT, 11 (CFR, 36.7%; 19.9–56.1%) died compared with 7 deaths among the 12 who did not receive DAT due to limited availability (58.3%; 27.7–84.8%) ($P = .17$).

Among 34 isolates available for MLST testing, a total of 7 STs and 4 isolates without ST identifications were found (Supplementary Table 1). Sequence types of 4 isolates were not identified because 1 allelic profile was not generated by the PubMLST database. ST-302 was the most common ST type (n = 17; 48.6%), followed by ST-67 (7 cases, 20%) and ST-458

Table 1. Comparison of the Characteristics of Patients Admitted to San Lazaro Hospital, Manila, the Philippines, With Clinically Diagnosed Diphtheria Between 1 January 2006 and 9 February 2017 According to Whether They Survived or Died

	Total, N or n (%)	Survived, n	Died, n	Mortality, %	*P*
Total[a]	267	150	117	43.8	
Sex					
Female	145 (54.3)	82	63	43.5	.902
Male	122 (45.7)	68	54	44.3	
Age group					
<5 years	83 (31.1)	33	50	60.2	<.01
5–9 years	103 (38.6)	57	46	44.7	
10–14 years	45 (16.9)	34	11	24.4	
15–19 years	23 (8.6)	19	4	17.4	
≥20 years	13 (4.9)	7	6	46.2	
DAT treatment given during the admission					
DAT not given	216 (80.9)	113	103	47.7	<.01
DAT given	51 (19.1)	37	14	27.5	

Abbreviation: DAT, diphtheria antitoxin.

[a]The observation period is 11.1 year between 1 January 2006 and 9 February 2017.

Table 2. Characteristics of Patients With Laboratory-Confirmed Diphtheria Admitted to San Lazaro Hospital, Manila, the Philippines, between 1 December 2015 and 9 February 2017 According to Whether They Survived or Died

	Total, N	Survived, n	Died, n	Mortality, %	*P*	RR (95% CI)
Total[a]	42	24	18	42.8		
Culture positive	35	19	16	45.7		
PCR positive (n = 34)	31	19	12	38.7		
Sex						
Male	17	9	8	47.1	.755	1.18 (.59–2.36)
Female	25	15	10	40		Ref
Age group						
<5 years	15	6	9	60	.154	2.34 (.82–7.04)
5–9 years	5	1	4	80		3.20 (1.08–9.48)
10–14 years	12	9	3	25		Ref
15–19 years	7	7	0	0		N/A
≥20 years	3	1	2	66.8		2.67 (.74–9.60)
Living area[b]						
Metro Manila	28	19	9	32.1	<.05	Ref
Outside Metro Manila	14	5	9	64.3		2.00 (1.03–3.89)
Duration of symptom						
1–4 days	25	18	7	28	<.05	Ref
≥4 days	17	6	11	64.7		2.31 (.12–4.75)
Underlying chronic disorders						
Any	5	1	4	80	.096	Ref
Not present	37	23	14	37.8		2.11 (1.16–3.86)
Number of DPT dose(s)						
0	11	9	2	18.2	.082	Ref
1 or 2	4	1	3	75		4.13 (1.03–16.6)
3	20	12	8	40		2.2 (.55–8.74)
Unknown/no record	7	2	5	71.4		3.93 (1.01–15.2)
DAT treatments given during the admission						
DAT given	30	19	11	36.7	.17	.62 (.32–1.23)
DAT not given	12	5	7	58.3		Ref

Abbreviations: CI, confidence interval; DAT, diphtheria antitoxin; DPT, diphtheria, pertussis and tetanus vaccine; N/A, not applicable; PCR, polymerase chain reaction; Ref, reference; RR, risk ratio.

[a]The observation period is 1.2 year between 1 December 2015 and 9 February 2017.

[b]San Lazaro Hospital is located in Metro Manila. Other data including symptoms, vital signs, and electrocardiogram findings are shown in Supplementary Table 2.

(5 cases, 14%) (Supplementary Table 1). One isolate with erythromycin resistance was of unknown ST type and the single locus variant of ST-457 and ST-40. Clonal analysis classified 7 clonal complexes among the 34 isolates including the 4 isolates without ST identifications. The phylogenetic tree and eBURST diagram did not show a clear relatedness among the 7 clonal complexes (Figure 1).

Case mapping showed a wide distribution of patients with diphtheria in Metro Manila (Figure 2). Many patients with diphtheria were identified in highly populated areas. Most of the laboratory-confirmed cases in 2015–2017 were resident in areas where patients with clinical diphtheria had been observed in the previous 11 years. Sequence types were scattered around the city, with no apparent clustering of ST types.

DISCUSSION

Our study showed that diphtheria persists as an important public health problem in Metro Manila. The disease is most common among children younger than 10 years, who also have the highest mortality. The introduction of enhanced laboratory capacity showed that laboratory-diagnosed cases occurred in areas where clinical diphtheria cases had been found in the previous 10 years. A variety of MLST types are circulating across the city, particularly in densely populated areas of the city.

We observed a high number of cases and high CFRs among children younger than 10 years. In 2016 and 2017 this was particularly marked in children younger than 5 years. National surveillance conducted by the DOH also reported a 20% increase in diphtheria cases in 2016 compared with cases observed in 2015 [10]. A contributing factor for this increased incidence in 2016 may be low vaccine coverage during 2014 and 2015. According to WHO and UNICEF (United Nations Children's Fund) reports, DPT3 coverage dropped from 89% in 2013 to 67% in 2014 and 60% in 2015, and this may have been due to national-level stockout of vaccine [9]. In most developed countries, the EPI led to rapid reductions in diphtheria incidence among children [3]. Toxigenic respiratory diphtheria among young children often occurs in areas where vaccine coverage

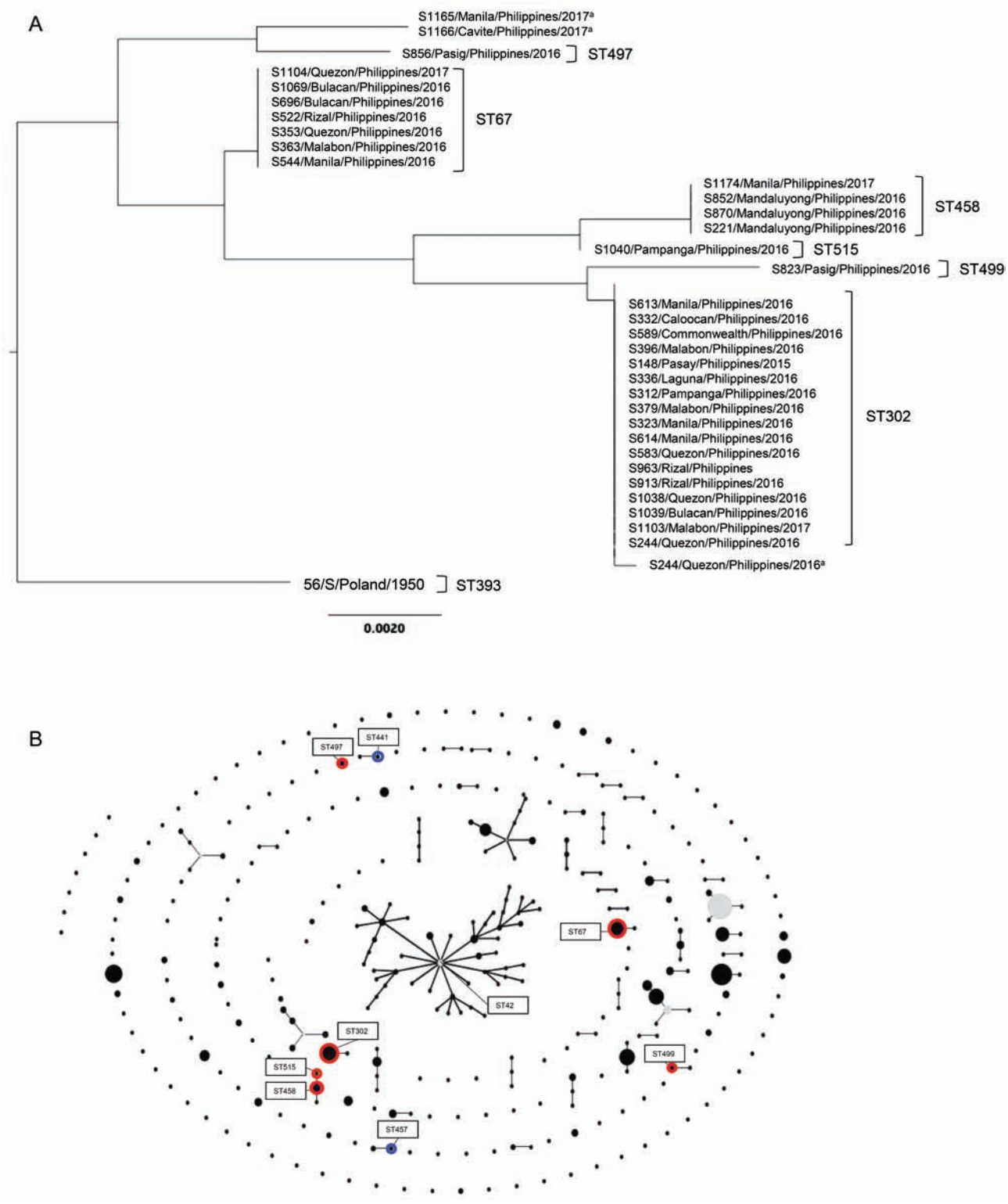

Figure 1. *A*, Phylogenetic tree of *Corynebacterium diphtheriae* isolates based on multilocus sequence types (STs) among patients admitted to San Lazaro Hospital (SLH) December 2015 and February 2017. [a]STs were not identified but S244/Quezon/Philippines/2016 is the single locus variant (SLV) of ST-302. S1165/Manila/Philippines/2017 and S1166/Cavite/Philippines/2017 were SLVs of ST-457. *B*, The eBURST diagram demonstrates the relatedness of isolates in this study and 703 isolates as reference STs from the multilocus sequence typing website (http://pubmlst.org/cdiphtheriae/, accessed 15 December 2018). The primary founder STs are shown in gray, and subgroup founder STs are shown in black. The STs isolated in our study and SVLs of unidentified STs in our study are indicated with red and blue halos, respectively.

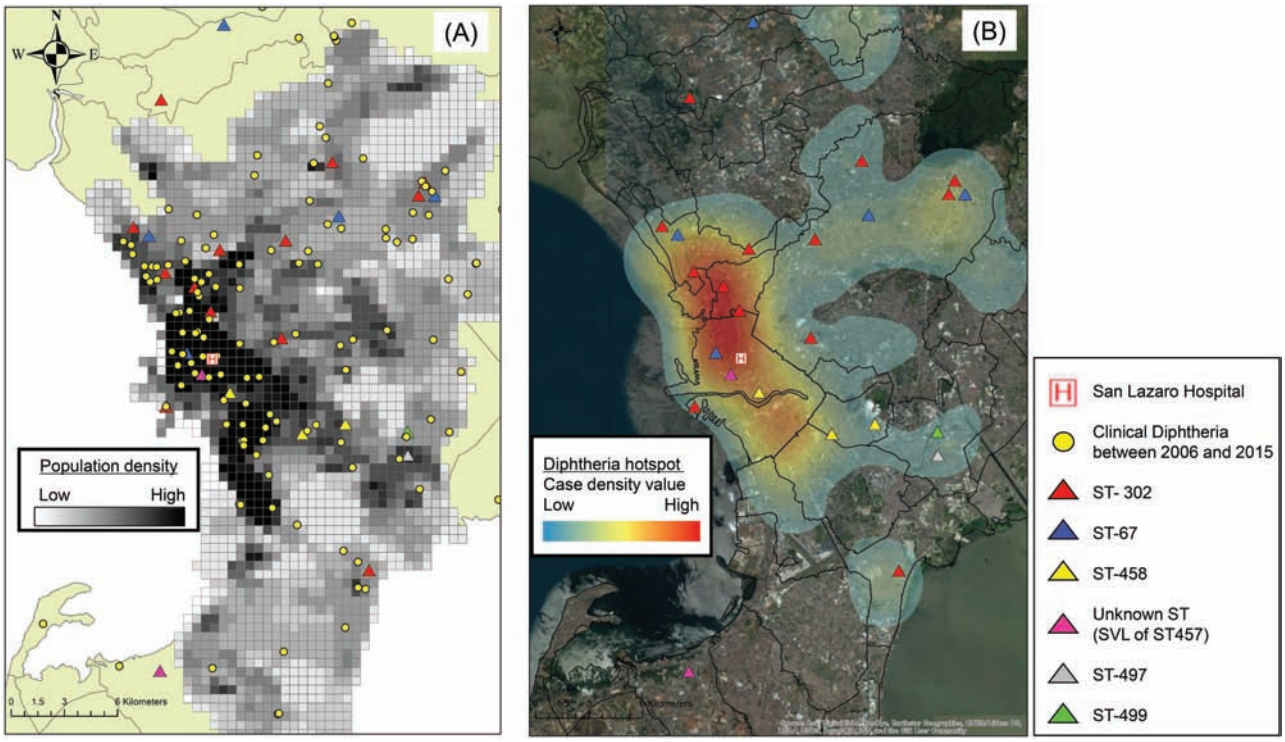

Figure 2. The spatial distribution of clinically and laboratory-confirmed diphtheria cases in Metro Manila. *A*, The home location of the clinically diagnosed diphtheria cases between 1 January 2006 and 30 November 2015 (yellow dots) and culture-positive diphtheria cases by the multilocus sequence type (ST) between 1 December 2015 and 9 February 2017 overlaid on a population-density map of Metro Manila. *B*, The home location of the culture-positive diphtheria cases by the multilocus STs between 1 December 2015 and 9 February 2017 overlaid on a hotspot map created using the clinically diagnosed diphtheria cases between 1 January 2006 and 30 November 2015. Abbreviation: SLV, single locus variant.

is not sufficient [3, 16]. These findings suggest the need to strengthen and sustain vaccine coverage in this area.

Several factors may have contributed to the higher CFR in our study compared with those of other reports [17–20]. This hospital is a tertiary-care medical institution, so many cases were severe by the time of referral. Milder cases are likely to have been treated in local health facilities, possibly without the clinical suspicion of diphtheria. Delayed treatment was observed in many cases and significantly associated with a higher CFR in our study. The CFR was significantly lower among DAT recipients compared with those without DAT treatment. The administration of DAT contributes to reducing mortality [21]. Early administration is critical because DAT is not able to neutralize the toxin once the toxin has entered host cells (particularly myocardial cells) [22]. DAT had not been reliably available in this hospital for the past 10 years. Since March 2016, the WHO provided DAT through the DOH in the Philippines [11]. A sustainable and secure supply of DAT needs to be maintained.

The CFR among those with a history of receiving 3 doses of primary vaccination course was not significantly lower than in those with no history of receiving the vaccine. Other studies have shown a strong association between unvaccinated status and death [23, 24]. The different result in this study may have been caused by misclassification of vaccine status due to recall bias. Only 8 patients were able to confirm their vaccine status with their immunization record card. Three patients were younger than 5 years and the others were aged 10–14 years. The finding of immunized individuals having severe toxigenic diphtheria in these age groups indicates the need to consider enhancements of a school-age booster but also a booster dose for children younger than 5 years.

Various molecular types of diphtheria were found in this study with a wide distribution across Metro Manila. There was no apparent clustering, although we observed overlapping distributions between patients with diphtheria and densely populated areas of Metro Manila (Figure 2). ST-302 is the major ST type in Manila, followed by ST-67 and ST-458. ST-302 and ST-67 were also found outside Metro Manila, and widely scattered distributions of the same ST inside Metro Manila were observed. The phylogenetic tree and eBURST diagram showed 7 distinct clonal complexes with no apparent clusters. In the MLST database, these ST types have also been reported in other continents (ST-302: Germany with a travel history to Malaysia; ST-67: France, Belgium; ST-458: Malaysia; ST-457: Malaysia; ST-497: Germany; ST499: Germany). Although our study was unable to compare molecular type with older isolates, it is possible that the same strains of diphtheria have been circulating for a long time in the area. One study reported an outbreak strain

circulating in the same area over decades [14]. The genome of *Corynebacterium* species is stable and genome rearrangements rarely occur [25, 26].

Our study has limitations. We studied patients attending a single hospital in Metro Manila, which may not reflect the true situation across the city. It is likely that the burden of disease is underestimated as mild cases may not be referred to this hospital. All laboratory-confirmed cases in this study were respiratory toxigenic diphtheria. Cutaneous diphtheria and chronic rhinitis (ozenae) were not included in this study and are probably underdiagnosed in this area. We did not identify the biotype of the isolates but consider that MLST is more helpful in understanding the epidemiology of the infection [27]. We performed tox-gene PCR but did not perform Elek`s test to determine the toxigenicity of the isolates. Tox-gene PCR alone is not able to provide a definitive confirmation of toxin production, but the bacteria were isolated from patients with the clinical picture of symptomatic respiratory diphtheria. E-tests are not the recommended method for MIC testing [15]. The diagnostic capacity for diphtheria in the Philippines is limited. A recent global survey on diphtheria diagnostic capacity conducted by the WHO showed that significant gaps in the capacity exist in the Western Pacific Region. The WHO Collaborating Center for Diphtheria recently conducted hands-on laboratory training for diphtheria to build capacity in laboratory diagnosis in the region [28]. To improve diphtheria surveillance, it is necessary to strengthen diagnostic capacity in the Philippines.

Our study revealed that diphtheria persists as a public health problem in Metro Manila, the Philippines, causing preventable childhood deaths. Renewed efforts to improve vaccine coverage of the primary course of DPT are needed, as well as consideration of an additional booster dose for children younger than 5 years, encouraging education and awareness for early detection and treatment, and ensuring a sustainable supply of DAT.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. N. S., V. O. D., and C. M. P. proposed the study idea. N. S., M. S., K. A., C. S., and C. M. P. contributed to the literature review. The study was designed by N. S., V. O. D., M. S., K. A., and C. M. P. Data collection was done by N. S., V. O. D., E. F. O. T., D. V. U., R. M. S., A. Q. D., F. D. G., and E. P. S. Data analysis was done by N. S., H. F., M. S., C. S., K. A., and C. M. P. Molecular analysis was done by N. S. and H. F. Figures were created by N. S., H. F., M. S., and C. M. P. All authors contributed to the writing of the final report.

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