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Investigation of a Large Diphtheria Outbreak and Cocirculation of *Corynebacterium pseudodiphtheriticum* Among Forcibly Displaced Myanmar Nationals, 2017–2019

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Supplementary Data

Supplementary materials are available at *The Journal of 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.

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Abstract

Background.—Diphtheria, a life-threatening respiratory disease, is caused mainly by toxin-producing strains of *Corynebacterium diphtheriae*, while nontoxigenic corynebacteria (eg, *Corynebacterium pseudodiphtheriticum*) rarely causes diphtheria-like illness. Recently, global diphtheria outbreaks have resulted from breakdown of health care infrastructures, particularly in countries experiencing political conflict. This report summarizes a laboratory and epidemiological investigation of a diphtheria outbreak among forcibly displaced Myanmar nationals in Bangladesh.

Methods.—Specimens and clinical information were collected from patients presenting at diphtheria treatment centers. Swabs were tested for toxin gene (*tox*)-bearing *C. diphtheriae* by real-time polymerase chain reaction (RT-PCR) and culture. The isolation of another *Corynebacterium* species prompted further laboratory investigation.

Results.—Among 382 patients, 153 (40%) tested *tox* positive for *C. diphtheriae* by RT-PCR; 31 (20%) PCR-positive swabs were culture confirmed. RT-PCR revealed 78% (298/382) of patients tested positive for *C. pseudodiphtheriticum*. Of patients positive for only *C. diphtheriae*, 63% (17/27) had severe disease compared to 55% (69/126) positive for both *Corynebacterium* species, and 38% (66/172) for only *C. pseudodiphtheriticum*.

Conclusions.—We report confirmation of a diphtheria outbreak and identification of a cocirculating *Corynebacterium* species. The high proportion of *C. pseudodiphtheriticum* codetection may explain why many suspected patients testing negative for *C. diphtheriae* presented with diphtheria-like symptoms.

Keywords

diphtheria outbreak; *Corynebacterium pseudodiphtheriticum* ; *Corynebacterium diphtheriae*

Respiratory diphtheria is a highly contagious disease caused mainly by the toxin-producing bacterium *Corynebacterium diphtheriae*. Diphtheria is characterized by sore throat, low-grade fever, and a firmly adherent grayish-white pseudomembrane over the nasopharyngeal, laryngeal, or tonsillar mucosa. In severe cases, gross cervical lymphadenopathy and soft tissue swelling gives rise to a bull neck appearance. Transmission occurs through respiratory droplets and close physical contact. Treatment includes the administration of diphtheria

antitoxin and antibiotics. The case fatality ratio for diphtheria is estimated at 29.0% in untreated and never-vaccinated cases, with the highest mortality rates in unvaccinated children aged < 5 years [1].

Following the introduction of diphtheria toxoid-containing vaccines in the 1940s–1950s, the incidence of diphtheria in industrialized countries rapidly declined. Although vaccination programs have been largely successful and diphtheria cases have declined worldwide, outbreaks continue to occur. Since 2010, several countries have reported large diphtheria outbreaks [2–5]. Low vaccine coverage rates resulting from the breakdown of health care infrastructure due to natural disasters, economic decline, and political conflict have contributed to the resurgence of outbreaks [6].

From August to December 2017, a massive influx of approximately 630 000 forcibly displaced Myanmar nationals (FDMNs) created makeshift settlements in and around established refugee camps in Bangladesh. [7]. In early November 2017, Médecins Sans Frontières (MSF) reported the first suspected case of diphtheria in a FDMN. By mid-December, MSF reported over 800 suspected cases through the World Health Organization (WHO) disease surveillance system in the area [8]. To confirm that *C. diphtheriae* was circulating among this population, the Bangladesh Institute of Epidemiology, Disease Control and Research (IEDCR) within the Ministry of Health and Family Welfare requested technical assistance from the Centers for Disease Control and Prevention (CDC) to implement testing for diphtheria. This report describes the epidemiology and laboratory investigations to confirm and characterize the diphtheria outbreak.

METHODS

Study Population Data and Specimen Collection

A convenience sample of patients presenting with symptoms suspicious of diphtheria to 2 MSF diphtheria treatment centers located in Balukhali and Jamtoli camps from 19 to 25 December 2017. From 9:00 am to 3:30 pm daily, trained technicians collected paired throat and nasal swabs from patients [9] and extracted patient data using a standardized form from clinical triage notes. Data collected included age, sex, and clinical symptoms documented by health care providers. Vaccination status was not available.

Suspected Disease Classification

Based on clinical symptoms and duration of illness, suspected diphtheria patients were classified as having mild, moderate, or severe disease. Mild disease was defined by an absence of pseudomembrane and either symptoms of sore throat, fever, or difficulty swallowing, or a diagnosis of tonsillitis, pharyngitis, or laryngitis, irrespective of duration. Moderate disease was defined as the same symptoms for mild disease, but with the presence of patchy pseudomembrane covering the nasopharynx or tonsils for a duration of 2 days or less. Severe disease was defined as the same symptoms for mild disease, but with the presence of extensive or patchy pseudomembrane covering the nasopharynx or tonsils, or presence of neck swelling, for a duration of 3 days or more.

Technology Transfer and Testing Locations

CDC scientists provided technical assistance to establish *C. diphtheriae* laboratory testing capacity at IEDCR. All throat swabs were processed at IEDCR. Due to limited laboratory capacity during the outbreak response, all nasal swabs were stored at −80°C, transported to CDC and tested from July to November 2018.

Laboratory Testing

Sterile flocked nylon swabs were used to collect throat and nasal specimens. Throat specimens were placed in Amies transport medium without charcoal (Becton, Dickinson, and Company). Nasal swabs were placed in silica gel sachets (Grace Davison). Specimens were packaged on site daily and transported overnight with cold packs to IEDCR.

Of the approximately 55 throat swabs collected daily, 20–30 were selected and processed within 48 hours of collection based on a proportional representation from each disease severity category; the remaining throat swabs were refrigerated (4–8°C) and processed within 2 weeks of the final collection day.

Throat and nasal swabs were cultured on trypticase soy agar with 5% sheep blood (TSA + SB; Thermo Fisher Scientific) and/or Tinsdale agar (Remel and BD) and incubated at 37°C. Tinsdale plates were examined at 24 and 48 hours for the presence of black colonies surrounded by diffuse brown staining (halos). Growth on TSA + SB was checked for small, white-gray colonies with little or no hemolysis. Colonies with appropriate morphology were subcultured on TSA + SB or Tinsdale agar. Isolates were screened by Albert stain (throat swabs) or Gram stain (nasal swabs), and identified with the API Coryne kit (bioMérieux). Toxigenicity was determined by the modified Elek test [10]. Limited culture media availability resulted in 73% (278/382) of throat swabs being cultured. All 382 nasal swabs were cultured at CDC.

Total nucleic acid (TNA) was extracted from throat and nasal swabs with a modified Qiagen DNA Mini extraction protocol (Qiagen) [11]. Throat or nasal swabs were placed in 1 mL of phosphate buffered saline or brain heart infusion broth, respectively, and mixed vigorously for 5 minutes. Swabs were removed and material was pelleted by centrifugation at 16 000*g* for 5 minutes. Supernatant was removed and pellets were resuspended in 180 μL Tris-EDTA buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). Lysozyme treatment and lysis incubations were performed as described previously, with the exception of the 70°C incubation shortened to 1 hour [12]. TNA was purified in QIAamp spin columns according to manufacturer's instructions. Columns were incubated at 70°C for 5 minutes in 200 μL elution buffer before TNA elution. A negative extraction control was processed with every 11 specimens. TNA was stored at 4°C and tested within 48 hours of extraction.

TNA extracts from specimens and extraction controls were tested in duplicate by a CDC real-time polymerase chain reaction (RT-PCR) *Corynebacterium* triplex assay targeting *tox*, *C. diphtheriae rpoB* gene, and *C. ulcerans*/*C. pseudotub erculosis rpoB* gene [12]. Each 20 μL reaction included 10 μL Quanta PerfeCTa qPCR Toughmix with UNG, low Rox (Quanta BioSciences), 2 μL template, and primer and probe concentrations as described in Williams et al [12]. Water served as a no-template control and was included between each specimen

on the PCR plate. PCR was performed in an Applied Biosystems 7500 Fast Dx instrument (Life Technologies). Cycling conditions included preincubation at 45°C for 10 minutes, denaturation at 94°C for 10 minutes, followed by 45 cycles of 94°C for 30 seconds and 60°C for 1 minute. Threshold was set at 0.2 and averaged Ct (cycle threshold) values less than 40 were considered positive for each target.

Presence of TNA in extracts was confirmed in nasal swabs with a separate RT-PCR assay targeting the human RNaseP gene. Primers and probe were included at 0.4 and 0.1 μM per reaction, respectively [13]. Enzyme and template amounts, as well as RT-PCR running conditions, were the same as in the *Corynebacterium* triplex assay.

Specimen TNA extracts were tested for the presence of *Corynebacterium pseudodiphtheriticum* by a previously published RT-PCR assay targeting the *rpoB* gene, with the same Quanta enzyme, instrument, and cycling conditions used for *Corynebacterium* triplex amplification. Positive results displayed an average Ct < 37, a cutoff previously set for this assay [14]. All *C. pseudodiphtheriticum* testing occurred after the primary investigation using the same assay at both laboratories.

For this analysis, specimens testing positive for *tox* were interpreted as *tox*-bearing *C. diphtheriae*. Culture followed by Elek testing was used to confirm the presence of toxigenic *C. diphtheriae* but was not used to classify positive cases overall due to limited culture capacity at IEDCR during the outbreak and 6 months' delay before analyzing nasal specimens.

Antimicrobial Susceptibility Testing Using Etest

The minimum inhibitory concentration (MIC) of amoxicillin, azithromycin, clarithromycin, clindamycin, daptomycin, erythromycin, levofloxacin, meropenem, penicillin, rifampin, and vancomycin was determined for selected isolates of *C. diphtheriae* and *C. pseudodiphtheriticum* using Etest (bioMèrieux) following the manufacturer's instructions with the following exceptions: bacterial suspension concentration in 0.85% saline was adjusted to a 0.5 McFarland standard. This suspension was inoculated using a polyester swab onto 2 Mueller Hinton Agar plates (150 mm diameter) with 5% sheep blood (Thermo Fisher Scientific). Six Etest strips were applied to 1 plate and 5 to the other after inoculation. Plates were incubated in ambient air at 35°C ± 2°C for 20–24 hours, and up to 48 hours if needed. Susceptibilities for clindamycin, daptomycin, erythromycin, meropenem, penicillin, rifampin, and vancomycin were interpreted using available criteria for *Corynebacterium* as described by the Clinical and Laboratory Standards Institute (CLSI) [15]. As clinical breakpoints are lacking for *Corynebacterium* species and amoxicillin, azithromycin, clarithromycin, and levofloxacin, these were not assigned an interpretive category. *Streptococcus pneumoniae* ATCC 49619 was used as quality control for all drugs, incubated with 5% CO_2 at 35°C ± 2°C for 20–24 hours.

Broth Microdilution

Reference broth microdilution (BMD) testing was performed on a subset of 10 *C. diphtheriae* and 10 *C. pseudodiphtheriticum* to confirm Etest susceptibility results for all drugs listed in the previous section. The 96-well BMD panels were prepared at CDC and

consisted of serial dilutions of each drug in cation-adjusted Mueller-Hinton broth (Difco, Becton Dickinson) supplemented with 4% lysed horse blood; there was a final total volume of 100 μL per well. The drug-broth dilutions were inoculated with 10 μL of standardized inoculum using a 95-pin inoculator assembly (Caplugs). CLSI M45 guidelines were used for BMD testing [15]. *Streptococcus pneumoniae* ATCC 49619 was used as quality control.

Data Analysis

Data were analyzed using SAS 9.4 (SAS Institute). Descriptive statistics were used to summarize and compare demographic and clinical characteristics of patients by PCR test status.

RESULTS

From 19 to 25 December 2017, 705 suspected diphtheria patients presented at the 2 diphtheria treatment centers and paired throat and nasal swabs were collected from 54% (383/705). Daily collection percentages ranged from 44% to 65% (Supplementary Figure 1). Among the 382 patients for whom clinical data were available, 46% were male. For patients with known age, 79% (299/378) were aged 15 years or younger, with the median age of 8 (interquartile range, 7–15) years. Sore throat (89%) and fever (86%) were the most common symptoms recorded. Pseudomembrane, a classic diphtheria sign, was reported in 79% of patients and 34% of patients were reported to have a swollen neck. Twenty percent of patients were classified as having mild illness, 33% as moderate, and 47% as severe (Table 1).

Overall, 40% (153/382) of patients tested positive for *tox*-bearing *C. diphtheriae* by RT-PCR; 67% (102/153) from throat swabs only, 13% (20/153) from nasal swabs only, and 20% (31/153) from both throat and nasal swabs. All specimens were negative for *C. ulcerans/C. pseudotuberculosis*. For confirmation of successful TNA extraction, human DNA was detected in all but 1 nasal swab. When considering laboratory results in conjunction with clinical data, patients testing RT-PCR–positive for *C. diphtheriae* (153/382) were more likely to be male (52%, 80/153), aged 15 years or younger (86%, 131/153), and 56% (86/153) were classified as having severe disease (Table 1).

Thirty-four *C. diphtheriae* isolates were recovered from throat and nasal swabs combined; 32 were toxigenic biovar mitis (25 throat and 7 nasal), and 2 (1 throat and 1 nasal) were nontoxigenic biovar gravis (Table 2). The 34 positive *C. diphtheriae* cultures were isolated from 31 patients; 3 patients had positive cultures from both nasal and throat swabs. Patient 1 had positive cultures for toxigenic *C. diphtheriae* mitis in both the throat and nasal swab; patient 2 was culture positive for toxigenic *C. diphtheriae* mitis from the throat swab and nontoxigenic *C. diphtheriae* gravis from nasal swab; and patient 3 was positive for toxigenic *C. diphtheriae* mitis from the nasal swab and nontoxigenic *C. diphtheriae* gravis from throat swab (Supplementary Table 1). All culture-positive patients (31/153) were positive for *tox*-bearing *C. diphtheriae* by RT-PCR (Supplementary Table 1).

Despite only 7 toxigenic *C. diphtheriae* isolates recovered from nasal swabs, there was an abundance of another putative *Corynebacterium* species evidenced by the presence of

black colonies without halos on Tinsdale media and diphtheroid cells observed with Gram stain. A convenience sample of 61 subcultures derived from nasal swabs were subsequently identified by API Coryne test strips as *C. pseudodiphtheriticum*. Molecular testing of all available specimens was then undertaken including all nasal swabs (382/382) and (379/382) throat swabs. RT-PCR identified 298 patients as positive for *C. pseudodiphtheriticum*: 1 from throat, 269 from nasal, and 28 from both throat and nasal swabs. Coinfection with *C. diphtheriae* and *C. pseudodiphtheriticum* was identified in 82% (126/153) of patients testing positive for *C. diphtheriae* (Table 3).

When analyzing all PCR-positive results for both *C. diphtheriae* and *C. pseudodiphtheriticum*, 27/153 patients tested positive for *C. diphtheriae* alone, 172 for *C pseudodiphtheriticum* alone, and 126 patients tested positive for both *Corynebacterium* species (Table 3). Fifty-seven patients tested negative for both *Corynebacterium* species (data not shown). Further stratification by RT-PCR targets showed a higher proportion of patients testing positive for *C. pseudodiphtheriticum* were classified as having mild illness (31%) when compared to *C. diphtheriae* (7%) or codetection (11%). A higher proportion of patients positive for only *C. diphtheriae* were classified as having severe disease (63%) when compared to patients with only *C. pseudodiphtheriticum* (38%) or codetection (55%) (Table 3).

Antimicrobial resistance testing was conducted by Etest on 34 *C. diphtheriae* and 56 *C. pseudodiphtheriticum* isolates. The susceptibility profiles for both *Corynebacterium* species were universally susceptible to meropenem, vancomycin, and daptomycin. Reduced susceptibility (MICs of 0.25–2 μg/mL) to penicillin was seen in 94% (32/34) of *C. diphtheriae* isolates tested and 3% (1/34) were resistant to both erythromycin and clindamycin (Table 4). *C. diphtheriae* isolates were susceptible to all other drugs with established breakpoints. Fifty-four percent (30/56) and 88% (49/56) of *C. pseudodiphtheriticum* isolates were resistant to erythromycin and clindamycin, respectively. Although there are no CLSI interpretive criteria available for azithromycin and clarithromycin for *Corynebacterium* species, *C. pseudodiphtheriticum* demonstrated the same MIC_{50} for clindamycin and azithromycin, 256 μg/mL. Eight of 10 *C. diphtheriae* isolates showed categorical agreement of Etest to the gold standard BMD method for all drugs with established breakpoints (CLSI M45 2015). Degree of categorical agreement between Etest and BMD results are described in Supplementary Table 2.

DISCUSSION

Our investigation confirmed that toxigenic *C. diphtheriae* biovar mitis was an important cause of cases presenting with diphtheria-like illness among a population of FDMNs. Nearly 90% of patients testing positive for *C. diphtheriae* were aged 15 years or younger. This finding reflects a characteristic epidemiologic feature of diphtheria in the prevaccine era where 70% of cases occurred in children younger than 15 years [16], suggesting the FDMN population was likely undervaccinated [17].

Furthermore, we identified cocirculation of another *Corynebacterium* species, *C. pseudodiphtheriticum*. *C. pseudodiphtheriticum* is generally considered part of the normal

human flora that colonizes the respiratory tract, skin, and mucous membranes [18]. In immunocompromised persons, it has been described as an opportunistic pathogen causing lower respiratory tract infections [19–22]. Although rare, there is evidence that *C. pseudodiphtheriticum* can cause diphtheria-like illness with exudative pharyngitis mimicking a diphtheria pseudomembrane [23–26]. The lack of diphtheria toxin in *C. pseudodiphtheriticum* suggests the pathogenicity and mechanism of disease is different from the toxin-mediated disease caused by *C. diphtheriae*. In addition, the carriage rate of *C. pseudodiphtheriticum* in the FDMN population, and in general, is unknown. Therefore, based on information from this investigation demonstrating that toxigenic *C. diphtheriae* was also circulating, it is not clear if we detected *C. pseudodiphtheriticum* carriage or infection.

Patients with *C. diphtheriae* were more likely to be classified as having severe disease compared to those only infected with *C. pseudodiphtheriticum*. This would be expected because diphtheria is toxin mediated and results in severe clinical disease. Of interest, patients coinfected with both organisms also had less severe disease than those only infected with *C. diphtheriae*. While this could be an artifact of a small sample size, other studies have suggested *C. pseudodiphtheriticum* is able to inhibit growth of other pathogenic bacteria, and therefore it may attenuate disease caused by *C. diphtheriae* [27, 28].

The *tox* gene was detected in 40% of our convenience sample, which is at odds with the high percentage of patients reported with pseudomembranes. There are several possible explanations for this discrepancy. Worldwide, few clinicians have seen a patient with diphtheria, making accurate identification of a characteristic pseudomembrane challenging. In addition, it is possible other pathogens apart from *C. pseudodiphtheriticum*, such as adenovirus, Epstein-Barr, herpes simplex virus, and *Candida albicans*, which can cause an exudative pharyngitis, were cocirculating. While testing for these pathogens was not available during the outbreak, it is interesting to note that an increase of upper respiratory infections with unknown etiology was reported beginning in August/September 2017 in the WHO Early Warning, Alert and Response System. Moreover, a new WHO case definition for suspected cases of diphtheria that included the presence of pseudomembrane was introduced on 11 December 2017, just prior to our investigation. The case definition change could have led to greater awareness of pseudomembranes, resulting in increased reporting during our data collection. From November 2017 to May 2018, the WHO reported only 35% of suspect patients had a pseudomembrane [29]. These data from a broader time frame are more aligned with our laboratory confirmation of 40%, suggesting that pseudomembranes were over reported during our investigation.

The PCR for the *tox* gene is 10 times more sensitive than the PCR test for the *rpoB* gene that is specific for *C. diphtheriae* [12]. The ability to detect the *tox* gene using RT-PCR resulted in 80% more patients testing positive for *C. diphtheriae* than had culture been used alone. Although RT-PCR is a helpful tool that can provide rapid results with increased sensitivity, culture followed by use of the modified Elek test is the gold standard for confirmation of toxin production in *C. diphtheriae* and related species. During this investigation, only 20% of RT-PCR *tox*-positive *C. diphtheriae* results were culture confirmed. There are several possible explanations as to why isolation rates were low. Identification of colonies with halos is subjective. Colonies with halos may have been missed, especially if there was heavy

overgrowth of other bacterial species, such as *C. pseudodiphtheriticum*. Also, there were reports of widespread antibiotic administration in the camps for presumed diphtheria and other illnesses. Therefore, prior use of antibiotics could have reduced the viable bacterial load in the pharynx and nasal passages contributing to negative culture results, especially if there were a several hour delay prior to patients reaching a diphtheria treatment center. Moreover, molecular diagnostics such as RT-PCR can detect the toxin gene in nonviable organisms. Long frozen storage of nasal swabs may have been an additional contribution to low *C. diphtheriae* recovery, but impact of each factor was not determined in this study.

Per WHO guidelines, recommended antibiotic treatment for diphtheria includes penicillin, erythromycin, and/or azithromycin. During this outbreak, treatment included penicillin or azithromycin. However, azithromycin has not traditionally been recommended as a treatment for diphtheria and the only evidence of effectiveness comes from in vitro data [30]. Greater than 90% of *C. diphtheriae* isolates tested in our investigation were intermediate to penicillin, and 3% were resistant to erythromycin, but they were susceptible to all other drugs tested with established breakpoints. *C. pseudodiphtheriticum* antimicrobial susceptibility results ranged from susceptible to highly resistant for erythromycin and clindamycin, which has been previously reported [18, 31–33]. The Etest method appeared to work well for testing *C. diphtheriae* for all drugs tested here. Comparison of Etest and BMD results for a limited subset of *C. diphtheriae* and *C. pseudodiphtheriticum* isolates demonstrated categorical agreement for most drugs (Supplementary Table 2). Among *C. pseudodiphtheriticum*, Etest tended to underestimate erythromycin MIC values in comparison to reference BMD, demonstrating the need for additional studies to assess its accuracy.

Although interpretive categories are not established for azithromycin against *Corynebacterium* species, *C. pseudodiphtheriticum* azithromycin MICs (μg/mL) were approximately 8500-fold greater than *C. diphtheriae* MICs in this outbreak. An azithromycin MIC of 256 μg/mL far exceeds the achievable azithromycin level in tonsillar tissue and assumed resistance by the *C. pseudodiphtheriticum* isolates to azithromycin therapy [34].

These antimicrobial susceptibility testing findings have implications for future outbreaks, especially when there is cocirculation of both *Corynebacterium* species: use of erythromycin or azithromycin could potentially select for continued transmission of *C. pseudodiphtheriticum*. There is also a need to better understand the effectiveness of azithromycin in treatment of diphtheria.

This investigation established *C. diphtheriae* laboratory testing capacity in Bangladesh and confirmed that toxigenic *C. diphtheriae* was circulating among the FDMN population, emphasizing that diphtheria remains a public health threat. Genomic studies comparing the relationship between isolates in this outbreak to others in the region are ongoing. Furthermore, we identified a high rate of cocirculating *C. pseudodiphtheriticum*, which may explain some of the diphtheria-like symptoms in patients testing negative for *C. diphtheriae*. Future studies are needed to understand the role of *C. pseudodiphtheriticum* carriage, its potential to cause diphtheria-like disease, and how azithromycin may have impacted the clinical presentations during this outbreak.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic and Clinical Characteristics of Suspected and Confirmed Diphtheria Patients Presenting to Diphtheria Treatment Centers, 19–25 December 2017

Characteristic	Total, No. (%)	*Corynebacterium diphtheriae*, No. (%)
No. persons	382 (100)	153 (40)
Sex[a]		
Male	175 (46)	80 (53)
Female	206 (54)	72 (47)
Age group, y[b]		
0–4	52 (14)	20 (13)
5–9	124 (33)	64 (43)
10–15	123 (32)	47 (31)
16–29	61 (16)	18 (12)
30+	18 (5)	1 (1)
Signs and symptoms		
Sore throat	341 (89)	140 (91)
Fever	329 (86)	131 (86)
Pseudomembrane	304 (79)	137 (89)
Swollen neck	132 (34)	58 (38)
Pseudomembrane and swollen neck	104 (27)	52 (34)
Disease categories		
Mild	78 (20)	16 (10)
Moderate	124 (33)	51 (33)
Severe	180 (47)	86 (56)

[a]Missing sex, 1 case.

[b]Missing age, 4 cases.

Table 2.

Corynebacterium diphtheriae Culture and Elek Test Results

	Throat (n = 382)				Nasal (n = 382)				
	Positive Cultures				Positive Cultures				
Test Results	**Mitis Biovar**	**Gravis Biovar[a]**	**Negative Cultures**	**Not Tested**	**Mitis Biovar**	**Gravis Biovar[b]**	**Negative Cultures**	**Not Tested**	**Total Throat/Nasal (n = 764)**
Toxigenic	25	…	…	…	7	…	…	…	32
Nontoxigenic	…	1	…	…	…	1	…	…	2
Not tested	…	…	…	104	…	…	…	…	104
Negative	…	…	252	…	…	…	374	…	626

No result is indicated by …

[a]Patient with nontoxigenic *C. diphtheriae* gravis from throat swab and toxigenic *C. diphtheriae* mitis by nasal swab.

[b]Patient with nontoxigenic *C. diphtheriae* gravis from nasal swab and toxigenic *C. diphtheriae* mitis by throat swab.

Table 3.

Demographic and Clinical Characteristics of Patients Testing Positive for *Corynebacterium diphtheriae*, *C. pseudodiphtheriticum*, and Coinfections of Both *Corynebacterium* Species by Real-Time PCR, 19–25 December 2017

Characteristics	*C. diphtheriae*, No. (%)	*C. pseudodiphtheriticum*, No. (%)	Codetection, No. (%)
No. persons (n = 382)	27 (7)	172 (45)	126 (33)
Sex[a]			
Male	9 (35)	74 (43)	71 (56)
Female	17 (65)	98 (57)	55 (44)
Age group, y[b]			
0–4	1 (4)	30 (18)	19 (15)
5–9	7 (27)	53 (31)	57 (46)
10–15	12 (46)	60 (35)	35 (28)
16–29	6 (23)	21 (12)	12 (10)
30+	0 (0)	7 (4)	1 (1)
Disease classification			
Mild	2 (7)	53 (31)	14 (11)
Moderate	8 (30)	53 (31)	43 (34)
Severe	17 (63)	66 (38)	69 (55)

The *C. diphtheriae* column includes only patients testing RT-PCR positive for *C. diphtheriae*.

C. pseudodiphtheriticum column includes only those testing RT-PCR positive for *C. pseudodiphtheriticum*. The codetection column includes only those testing RT-PCR positive for both *Corynebacterium* species.

n = 57 patients tested negative by RT-PCR for both *Corynebacterium* species.

Abbreviation: RT-PCR, real-time polymerase chain reaction.

[a]Missing sex, 1 case.

[b]Missing age, 4 cases.

Table 4.

Antimicrobial Susceptibilities of 34 *Corynebacterium diphtheriae* Isolates and 56 Nasal Isolates of *Corynebacterium pseudodiphtheriticum* Determined by Etest

Antimicrobial	MIC_{50}, µg/mL		MIC_{90}, µg/mL		Intermediate, %		Resistant, %	
	C. dip	C. pd	C. dip	C. pd	C. dip	C. pd	C. dip	C. pd
Penicillin	0.25	0.008	0.5	0.016	94	0	0	0
Amoxicillin	0.5	0.03	0.5	0.03	NI	NI	NI	NI
Meropenem	0.06	0.004	0.06	0.008	0	0	0	0
Vancomycin	1	0.5	1	1	NA	NA	NA	NA
Daptomycin	0.125	0.06	0.125	0.06	NA	NA	NA	NA
Azithromycin	0.03	256	0.06	256	NI	NI	NI	NI
Erythromycin	≤0.016	2	≤0.016	4	0	27	3	54
Clarithromycin	≤0.016	4	≤0.016	16	NI	NI	NI	NI
Levofloxacin	0.125	0.5	0.125	32	NI	NI	NI	NI
Clindamycin	0.125	256	0.25	256	0	0	3	88
Rifampin	0.002	≤0.002	0.004	2	0	11	0	4

Abbreviations: C. dip, *C. diphtheriae*; C. pd, *C. pseudodiphtheriticum*; MIC_{50}, 50% minimum inhibitory concentration; NA, no intermediate or resistant category defined by CLSI M45 2015 guidelines for this drug for *Corynebacterium* spp., all isolates were susceptible to tested drug; NI, no interpretive category available using CLSI M45 2015 guidelines.