TOXIGENIC DIPHTHERIAE PROFILE IN CHILDREN IN 2017-2018

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ABSTRACT

Corynebacterium diphteriae is divided based on its ability to produce toxins. Toxigenic *C. diphteriae* is the type that has the capacity to produce toxins and is life-threatening. A diagnostic test used to confirm diphtheria infection is to culture, isolate, identify, and measure bacteria toxigenicity. This study was a descriptive study of *Corynebacterium* diphteriae found in children less than 18 years old who were administered to National Infection Center Prof. Dr. Sulianti Saroso. Descriptive analysis has been done to recognize characteristics, risk factors, signs and symptoms, and complications. We found 36 viable toxigenic *C. diphteriae* isolates, 52.8% intermedius strain, 33.3% gravis strain, 8.3% mitis, and 5.6% non-strain. Most of the hosts (52,8%) were 60-144 months years old. The majority of the host had completed the basic vaccination recommended by the Indonesian Paediatrician Association, while 75% of them hadn't gotten the supplementary doses. Fever (48.5%) and odynophagia (54.5%) were experienced by toxigenic intermedius strain infection. Snoring (50%) and thick pseudomembrane was mostly found in the gravis strain host, while the Bullneck sign was found in intermedius strain infection. Complications like airway obstruction, chronic kidney disease, and myocarditis were found in hosts with toxigenic intermedius strain by 66.7%, 100%, and 75%. Death cases were also mostly with this strain. Intermedius strains were mostly found in this study. Second dose vaccination related to diphtheria morbidity. Fever and painful swallowing were symptoms of intermedius strain infection. Complications and mortality were also connected to intermedius strain infection.

Keywords: *Corynebacterium Diphteriae*; Strain; Toxigenic

INTRODUCTION

Corynebacterium diphtheriae is a causative agent to diphtheria diseases. Pathogenic bacteria cause upper respiratory infections. This microorganism is the positive gram, facultatively anaerobic, rod-shaped, 1-8 µm in length, 0.3-0.8 µm in width size, and has no capsule and spore. The microorganism is non-motile and able to produce toxins.[1] Toxigenic biotype has the rule for natural boosting and defending population immunity to infection.

According to its morphology and biochemistry (including metabolism), infectious toxigenic *Corynebacterium diphtheriae* has three strains: gravis, intermedius, and mitis. Its virulence depends on the ability (quality and quantity) to produce toxins and growth rate. The range of time for each strain to self-reproduce (*in vitro*) is 60 minutes for the gravis strain, 100 minutes for the intermedius strain, and 180 minutes for the mitis strain while *in vivo.* The predicted growth rate is more rapidly done with the help of Fe ion as a catalysator. The toxin causes inflammation and epithelial destruction. This mechanism leads to tissue necrosis, pseudomembrane, and mucous tissue edema. As a result, the upper respiratory tract occurs. The toxin can travel via the bloodstream and causes

degeneration and necrosis, mainly in heart and nerve cells. Mortality is primarily induced by heart and/or respiratory failure.[2]

Transmission occurs via droplet with an incubation time of 2-7 days after getting infected. Humans, as the host, could show clinical manifestations or be carriers.[3] Recent studies showed the infection involved adults, not only children, as we've known before. Diphtheria endemic could repeatedly happen by decreasing vaccination coverage, bacterial virulence change, and carrier or patient from endemic region.[4,5]

From 2000-2015, the Southeast Asia region had always been the first region with the most confirmed cases of diphtheria every year. Indonesia is in second place after India. From 2011-2015, 3203 cases were confirmed in Indonesia, while India had 18.350 cases.[6]
Diphtheria has been decided as an outbreak case in Indonesia recently. In East Java, it was reported 11 children passed away out of 333 diphtheria cases.[7] Following by sequential years, from 2012 until 2016, the mortality rate was 1192, 778, 396, 252, and 340 cases in adults and children. In 2016, DKI Jakarta province notified mortality rate was 1 out of 4 patients, while Banten reported no cases.[8] The declaration of rapidly increasing number occurred in 30 provinces, 170 cities/districts with 939 cases and 44 deaths in 2017.[9] At National Infection Center Prof. Dr. Sulianti Saroso, the mortality rate had also increased for three years, two deaths out of 20 cases in 2016, 3 deaths out of 140 cases in 2017 and 9 fatal cases out of 177 cases in 2018.

The specimens collected from inpatient cases in National Infection Center Prof. Dr. Sulianti Saroso were sent to a national laboratory owned by Health Ministry, Infectious Diseases Centre- Health Primary Biomedical and Technology. One of the alternative examinations to detect the toxigenicity of *C.diphtheriae* is the Polymerase Chain Reaction technique. The main purpose of this study was to learn demographic characteristics, risk factors, signs, and symptoms, as well as complications and output of the host.

MATERIAL AND METHODS

Study Design and Setting

The research was a descriptive study on isolating children with confirmed diphtheria cases in hospitalized patients of Sulianti Saroso Infectious Diseases Hospital (aged ≤ 18 years old) from January 2017 until December 2018. We had a total sample of 36 patients that completed the data variable (Figure 1). Data collection was taken from the medical record and registered laboratory results. The Ethics Committee approved the study protocol at the National Infection Centre by Prof. Dr. Sulianti Saroso, number: 21/XXXVIII.10/V/2018.

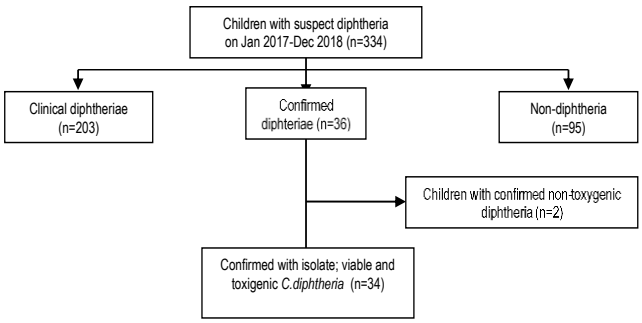

Figure 1. Flow of subject

Sampling and Statistical Methods

Inclusion criteria were toxigenic *Corynebacterium diphtheriae* isolate by Naso-oropharyngeal swab in children on January 2017 – December 2018 administered to National Infection Centre Prof. Dr. Sulianti Saroso. Data were collected by a scientist using a case report form. Descriptive analysis was done by SPSS, v.20 (SPSS Inc, Chicago, Illinois, USA).

RESULT

Thirty-six toxigenic *C. diphteriae* isolates that fulfilled inclusion criteria were collected out of 340 isolates examined by PCR technique. The microorganism wasn't found in 303 isolates, and the non-toxigenic one was found in the other isolate. Thirty-four (52,8%) isolates contained intermedius strain, 33.3% had gravis strain, 8.3% other had mitis strain, and 5.6% got no strain. Host characteristics identified gender, age, and domicile, as shown in Table 1.

Table 1. Characteristics of Confirmed Diphteriae

Host Characteristic		N	%
Gender	Male	19	52.8
	Female	17	47.2
Age	<12 months	0	0
	12-24 months	0	0
	>24-60 months	9	25.0
	>60 months -12 years	19	52.8
	>12 years	8	22.2
Living	Jakarta	21	58.3
	Other Jakarta	15	41.7
Vaccination	Completed	7	19.4
	Completed basic vaccination, an incomplete booster vaccination	17	47.2
	Completed basic vaccination	1	2.8
	Unvaccinated	1	2.8
	Unknown	10	27.8
Contact history	Yes	11	30.6
	No	9	25.0
	Unknown	16	44.4
Varian/Strain	Intermedius	19	52.8
	Mitis	3	8.3
	Gravis	12	33.3
	Non-Toxigenic / Non-Strain	2	5.6

Risk factors were vaccination status and contact history with a previously confirmed case. Most of the hosts in the study had completed the basic vaccination recommended by the Indonesian Paediatrician Association but not the booster dose (47,2%). Vaccination status is described in Table 1.

Typical symptoms identified in the infected host in our study were fever, painful swallowing, and snoring. Fever (48,5%) and painful swallowing (54.5%) were experienced by the host with intermedius strain, while snoring corresponded to all strains, as depicted by Table 2.

Table 2. Symptom and Clinical of Confirmed Diphteriae

Variable	Intermedius N=19	Mitis N=3	Gravis N=12
Fever			
Yes	16 (48.5%)	3 (9.1%)	12 (36.4%)
No	3 (100%)	0 (0%)	0 (0%)
Painful swallow			
Yes	18 (54.5%)	2 (6.1%)	11 (33.3%)
No	1 (33.3%)	1 (33.3%)	1 (33.3%)
Snoring			
Yes	1 (25.0%)	1 (25.0%)	2 (50.0%)
No	18 (56.3%)	2 (6.3%)	10 (31.3%)
Pseudomembrane			
Yes	8 (38.1%)	3 (14.3%)	9 (42.9%)
No	11 (73.3%)	0 (0%)	3 (20.0%)
Bullneck			
Yes	6 (54.5%)	1 (9.1%)	3 (27.3%)
No	13 (52.0%)	2 (8.0%)	9 (36.0%)

Clinical signs were bleeding-susceptible pseudomembrane in the gravis strain (42.9%) and bullneck in the intermedius strain (54.5%), as studied in Table 2. Majority of the complication afflicted the host with intermedius strain; airway obstruction, chronic kidney failure, secondary infection, and myocarditis. Mortality cases were found in intermedius strain (83,3%) and non-strain host (16,7%) (Table 3).

Table 3. Complication and Outcome

Variable	Intermedius N=19	Mitis N=3	Gravis N=12
Airway obstruction			
Yes	4 (66.7%)	0 (0%)	2 (33.3%)
No	15 (50.0%)	3 (10.0%)	10 (33.3%)
Neuropathy			
Yes	0 (0%)	0 (0%)	0 (0%)
No	19 (100%)	3 (100%)	12 (100%)
Chronic kidney failure			
Yes	1 (100%)	0 (0%)	0 (0%)
No	18 (51.4%)	3 (8.6%)	12 (34.3%)
Secondary infection			
Yes	7 (77.8%)	0 (0%)	2 (22.2%)
No	12 (44.4%)	3 (11.1%)	10 (37.0%)
Myocarditis			
Yes	6 (75.0%)	0 (0%)	1 (12.5%)
No	13 (46.4%)	3 (10.7%)	11 (39.3%)
Outcome			
Death	5 (83.3%)	0 (0%)	0 (0%)
Alive	14 (46.7%)	3 (10.0%)	12 (40.0%)

DISCUSSION

The accurate clinical specimen was a fundamental diagnostic tool to confirm the disease. A recent approach by Efstratiou et al. stated that all probable diphtheria cases should have taken a nasopharyngeal and/or oropharyngeal swab. The diphtheria surveillance guideline released by World Health Organization in 2018 recommended naso-oropharyngeal swabs in suspected cases of the disease with direct contact.[10]

The study found 36 toxigenic *C.diphtheriae* out of 334 samples examined. Intermedius strain was recognized in 52,8% of samples, followed by gravis strain (33,3%), mitis strain (8,3%), and non-strain (5.6%). The result was parallel with an outbreak study in Maranhao Brasil in 2012, which concluded that the intermedius strain was the causative agent. This finding contradicts a study conducted by Soegianto in East Java province, which stated the gravis and mitis strains as the majority causative agent,[11] likewise the outbreak in East Europe in 2015.[12]

Migration factors from endemic regions to other regions, vaccination coverage, and population immunity influenced *C.diphtheriae* biotype distribution change.[13,14] Population immunity to specific biotypes could minimize the biotype circulation in the future.[14]

When analyzing the gender proportion, the study found more men than women, as some studies explained women had a higher risk of diphtheria infection because they had lower immunity levels than men.[1] Therefore, women are more likely to be attacked by diphtheria.[15] Children under 18 years old participated in this study;> 60 – 144 months old covered 52,8% participants, >24-60 months old as 25%, and >144 months old as 22,2%. Diphtheria mainly attacked 0-24 months old children. However, vaccination

coverage in this group was much higher than in the older group. As a result, there has been a trend shifting to preschool and student groups worldwide, including Indonesia.[16] A study in India resulted in the same outcome. It indicated vulnerability to *C. diphtheriae* infection. This phenomenon was caused either by poor diphtheria vaccination coverage or decreasing natural or artificial immunity to diphtheria.[17]

The poor outcome of the host in our study was the result of a low booster vaccination. The issue was similar to a study reported by Meshram, which described more than 57.4% were partially vaccinated.[18] The Nawing study showed a significantly associated lack of basic or booster vaccination.[19] The key to avoiding infection of *C.diphtheriae* in children is simultaneous improvement in their nutritional status and completed vaccination.

Clinical manifestations were noted in medical records. Fever, sore throat, and swollen neck, similar to goiter in appearance, were found in the majority. The usual symptoms experienced by diphtheria patients are high body temperature, occasionally shivering, followed by sore throat, difficulty swallowing food, nausea, vomiting, malaise, swollen neck similar to goiter, and a dirty state of the mouth cavity.[1] All patients in our study presented with fever, a temperature of 38° C (100.4°F) or below as clinical symptoms of diphtheria. The symptoms usually lasted between two until seven days after infection.[3] Bleeding-susceptible pseudomembrane and goiter-like bullneck had been met in the host with intermedius strain. Subfebrile condition was described in early-onset infection.[20] Pseudomembrane was built as the result of fibrin matrixes, bacteria, dead epithelial tissue, and inflammatory cells that strongly adhered to the tissue. The disease severity was related to expansive local infection; nevertheless, the pseudomembrane rule in keeping *C.diphtheriae* viability hadn't been clearly investigated. Diphtheria toxin was considered the main factor responsible for tissue defect and fibrin production. Bacteriophage was in charge as an important rule to bring diphtheria toxin gen (tox) to form pseudomembrane. It was reported that coagulase *C.diphtheriae* activity binds to fibrinogen and reformed fibrinogen to fibrin to shape pseudomembrane. This enzyme also acted as a virulence determinant, either toxigenic or non-toxigenic species.[12]

Complications such as airway obstruction, chronic kidney failure, secondary infection, and myocarditis have shown up in this study. They appeared in the host with intermedius strain. Myocarditis also occurred in a patient with gravis strain, and non-strain contradicted to host with mitis one. Secondary infection explained in this research was dengue hemorrhagic fever and urinary tract infection that indicated toxigenic strain could cause systemic complication. Other possible pavement reported was myocarditis and polyneuropathy.[2,21] Myocarditis usually happens by the second week after onset, while polyneuropathy is in the third. Polyneuropathy influences either the motoric or sensory nervous system.[4] The damaged epithelial cells acted as port d'entrée and led to the bloodstream circulating and infecting other organs such as the heart, peripheral nerve, and central nervous system.[22] Life-threatening conditions were acute kidney injury, hypotension, and pneumonia.[3] Mortality cases tended to be found in hosts with intermedius strain (83.3%) and non-strain (16,7%). A study in the Georgia Republic reported 13% out of 127 hosts with gravis strain lead to death cases.[23] A research conducted by Lumio stated gravis biotype was more prone to lead to severe complications, while a recent study clarified that other toxigenic strains could cause death.[24] The underlying soft tissue edema and cervical adenitis could be intense, and particularly in children, smaller airways had more chance to induce airway obstruction and bull-neck appearance. In both adults and children, a common cause of death is suffocation after aspiration of the membrane.[3] Recently, the correlation between toxigenicity and drug resistance was not light. Invasive characteristics and toxigenicity have been proven to have no relation.[25,26]

CONCLUSION

This study showed that the intermedius strain could be easily found in hosts, both in boys and girls>60-144 months old in Jakarta. Uncompleted booster vaccination and direct contact with the previously confirmed case seemed to be the risk factor for infection. Classical symptoms found in the intermedius strain host were fever and dysphagia, while snoring was discovered in other strains. Bleeding-susceptible pseudomembrane was found to be an atypical sign in toxigenic Corynebacterium diphtheriae infection. Complications were airway obstruction, chronic kidney failure, secondary infection, and myocarditis. They were prone to toxigenic intermedius strain. Death was found in 83,3% of the host with intermedius strain and 16.7% in non-strain.

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