Data Article

Whole-genome sequencing data of *Corynebacterium diphtheriae* isolated from diphtheria outbreaks in Indonesia

Vivi Setiawaty\(^a,b\), Nelly Puspandari\(^b,e\), Ratih Dian Saraswati\(^b,e\), Dwi Febriyana\(^b,e\), Tati Febriantri\(^b,e\), Yuni Rukminiati\(^b,e\), Fauzul Muna\(^b,e\), Fitriana Fitriana\(^c\), Dodi Safari\(^c\), Rahadian Pratama\(^d\), Lisa Andriani Lienggonegoro\(^b,c\), Sunarno Sunarno\(^b,c,*\)

\(^a\) National Referal Infectious Diseases Hospital Prof. Dr. Sulianti Saroso, Jakarta, Indonesia
\(^b\) Centre for Research and Development of Biomedical and Basic Health Technology, National Institute of Health Research and Development (NIHRD), Ministry of Health, Jakarta, Indonesia
\(^c\) National Research and Innovation Agency (BRIN), Jakarta, Indonesia
\(^d\) Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia
\(^e\) Centre for Health Resilience and Resource Policy, Health Policy Agency, Jakarta, Indonesia

**ARTICLE INFO**

**Article history:**
Received 1 May 2022
Revised 22 June 2022
Accepted 5 July 2022
Available online 14 July 2022

**Keywords:**
*Corynebacterium diphtheriae*
Whole-genome sequencing
Diphtheria outbreak
Indonesia

**ABSTRACT**

*Corynebacterium diphtheriae* (*C. diphtheriae*) is the causative agent of diphtheria. The main virulence factor of *C. diphtheriae* is diphtheria toxin, which is encoded by the *tox* gene and regulated by the *dtxR* gene. The *tox* and *dtxR* genes are used as genetic markers to identify bacteria causing diphtheria by PCR. Here, we present the whole-genome sequencing (WGS) data of 18 *C. diphtheriae* isolates from diphtheria outbreaks in different regions in Indonesia. We used these data to identify single nucleotide polymorphisms (SNPs) associated with the *tox* and *dtxR* genes to verify the accuracy of the PCR assay and performed molecular typing with a multilocus sequence typing (MLST) approach. The data can be used for further analyses, such as antimicrobial resistance and bacterial virulence factors.

DOI of original article: [10.1016/j.mimet.2021.106198](https://doi.org/10.1016/j.mimet.2021.106198)

\(*\) Corresponding author at: Centre for Research and Development of Biomedical and Basic Health Technology, National Institute of Health Research and Development (NIHRD), Ministry of Health, Jakarta, Indonesia.

E-mail address: sunarno.1@brin.go.id (S. Sunarno).

https://doi.org/10.1016/j.dib.2022.108460

2352-3409/© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license ([http://creativecommons.org/licenses/by/4.0/](https://creativecommons.org/licenses/by/4.0/))
Specifications Table

| Subject | Biological Sciences |
|---------|---------------------|
| Specific subject area | Genomics |
| Type of data | Genome sequences data (DNA-seq raw reads) and table |
| How data were acquired | Illumina MiSeq sequencing platform (Illumina, San Diego, USA) |
| Data format | Raw sequences (FASTQ) and isolates data |
| Description of data collection | DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA was quantified by Qubit and nanodrop for purity. Libraries were prepared using the Nextera XT DNA library prep kit (Illumina, San Diego, USA). Sequencing was performed using the Illumina MiSeq system. |
| Data source location | Research Laboratory for Infectious Diseases, NIH RD, Ministry of Health, Jakarta, Indonesia |
| Data accessibility | Repository name: DNA Data Bank of Japan |
|                     | Data identification number (permanent identifier): PRJDB12216 |
|                     | Direct link to dataset: [https://ddbj.nig.ac.jp/resource/bioproject/PRJDB12216](https://ddbj.nig.ac.jp/resource/bioproject/PRJDB12216) |
| Related research article | Sunarno, Khariri, F. Muna, K. sariadji, Y. Rukminiati, D. Febriyana, T. Febrianti, R.D.Saraswati, I. Susanti, N. Pusandari, A. Karuniawati, A. Malik, A. Soebandrio. New Approach for the Identification of Potentially Toxigenic Corynebacterium sp. Using A Multiplex PCR Assay, J Microb. Meth. 184 (2021) 106198. [https://doi.org/10.1016/j.mimet.2021.106198](https://doi.org/10.1016/j.mimet.2021.106198) |

Value of the Data

- The whole-genome sequencing data of *Corynebacterium diphtheriae* isolated from Indonesia, including strains with sequence types that may originate from Indonesia provide insight on genetic diversity of *Corynebacterium diphtheriae*.
- This data can be analyzed by researchers to understand the molecular epidemiology of this pathogen, especially the molecular typing of some *Corynebacterium diphtheriae* isolated from Indonesia.
- These data provide DNA sequences of *Corynebacterium diphtheriae* as reference sequences to develop and verify molecular methods and can be used for further analyses, such as bacterial virulence factors and antimicrobial resistance.

1. Data Description

*Corynebacterium diphtheriae* is a causative agent of diphtheria, an acute infectious disease that usually attacks the upper respiratory system. Diphtheria is characterized by the formation of a distinctive pseudomembrane around the tonsils with several complications, including respiratory obstruction, myocarditis, and neuropathy [1]. The main virulence factor of *C. diphtheriae* is diphtheria toxin, an exotoxin that is responsible for the clinical manifestation and mortality of diphtheria. This toxin is encoded by the *tox* gene and regulated by the *dtxR* gene. The *tox* gene is carried by certain bacteriophages that are inserted into the bacterial chromosome by lysogenesis; therefore, the *tox* gene is only present in the toxigenic type (capable of producing diphtheria toxin) of *C. diphtheriae*. Meanwhile, the *dtxR* gene is found in *C. diphtheriae*, which can be both toxigenic and nontoxigenic [2]. Occasionally, there are some ‘anomaly’ types, known as
nontoxicogenic tox gene bearing (NTTB) types. In the NTTB type, the tox gene is present, but diphtheria toxin is not synthesized phenotypically and is grouped as a nontoxicogenic type [3].

The tox and dtxR genes are commonly used in laboratory tests for diphtheria using PCR assays. We sought to develop PCR assays with the tox and dtxR genes as targets for species identification and toxigenicity, including predicting 2 types of NTTB, resulting in an improved method [4]. Here, we present the whole-genome sequencing (WGS) data of 18 C. diphtheriae isolates from Indonesia (Table 1). The isolates were collected since 2012 until 2015, mostly have mitis subtype (61%). We used these data to identify SNPs associated with the tox and dtxR genes to verify the accuracy of the PCR assay [4]. We also used these data for molecular typing using the MLST approach [5]. All isolates were tested positive in Elek test and PCR tox gene, except ind_28 isolate which is the Sequence Type still not determined yet.

WGS data (FASTQ format) of 18 C. diphtheriae isolates have been deposited on DNA Data Bank of Japan (DDBJ) with data identification number: PRJDB12216 (https://dxbj.nig.ac.jp/resource/bioproject/PRJDB12216). These data could be used for further analysis regarding antimicrobial resistance and bacterial virulence factors.

2. Experimental Design, Materials and Methods

2.1. Isolate Collection and DNA Extraction

Eighteen C. diphtheriae were isolated from diphtheria outbreaks in Indonesia from 2012 to 2015 (Table 1). These isolates were randomly selected from Prof. Dr. Sri Oemijati Research Laboratory for Infectious Diseases, Jakarta as one of national reference laboratories. The isolates were obtained from clinical sample of diphtheria cases and their close contacts in some provinces of Indonesia. The archived C. diphtheriae isolates were stored by using TSB + 20% glycerol preservation medium in the ultra-low temperature freezer (-70 to -80 °C). The isolates were revived on blood agar plates and incubated at 37°C overnight. Bacterial species, biotype, and toxigenicity identification were performed by API Coryne (bioMérieux, La Balme les Grottes, France) and Elek tests according to WHO guidelines [6]. One full loop of bacterial colonies was dissolved in 500 μL of Ultrapure DNase/RNase-Free distilled Water (Invitrogen, Waltham, MA, USA). DNA

| No | Sample ID | Isolated Year | Subtype | Elek test | PCR tox gene | Sequence Type |
|----|-----------|---------------|---------|-----------|--------------|---------------|
| 1  | ind_02    | 2014          | mitis   | positive  | positive     | ST535         |
| 2  | ind_08    | 2014          | mitis   | positive  | positive     | ST535         |
| 3  | ind_24    | 2014          | gravis  | positive  | positive     | ST534         |
| 4  | ind_25    | 2015          | mitis   | positive  | positive     | ST534         |
| 5  | ind_26    | 2014          | mitis   | positive  | positive     | ST535         |
| 6  | ind_27    | 2015          | mitis   | positive  | positive     | ST535         |
| 7  | ind_28    | 2014          | gravis  | negative  | negative     | ND            |
| 8  | ind_34    | 2015          | mitis   | positive  | positive     | ST534         |
| 9  | ind_35    | 2012          | mitis   | positive  | positive     | ST377*        |
| 10 | ind_37    | 2013          | mitis   | positive  | positive     | ST377         |
| 11 | ind_42    | 2015          | mitis   | positive  | positive     | ST302         |
| 12 | ind_43    | 2015          | intermedius | positive | positive     | ST377         |
| 13 | ind_44    | 2015          | intermedius | positive | positive     | ST377         |
| 14 | ind_45    | 2015          | mitis   | positive  | positive     | ST534         |
| 15 | ind_46    | 2015          | mitis   | positive  | positive     | ST534         |
| 16 | ind_47    | 2015          | intermedius | positive | positive     | ST377         |
| 17 | ind_48    | 2015          | intermedius | positive | positive     | ST534         |
| 18 | ind_49    | 2015          | gravis  | positive  | positive     | ST534         |

ND=not determined
* The data have not been published
isolation was conducted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. In the last step, the DNA sample was stored in 50 μl of Ultrapure DNase/RNase-Free distilled Water (Invitrogen, Waltham, MA, USA). The DNA purity was measured using NanoDrop based on the 260/280 nm absorbance value with a ratio of 1.8–2.0. Quantification of DNA was conducted using a Qubit®3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA extraction was repeated when the quality or quantity of DNA did not meet the Illumina MiSeq platform requirements.

2.2. DNA Library and Whole-Genome Sequencing

DNA libraries were prepared using the Nextera XT DNA Library Prep Kit 2 × 150 bp (Illumina, San Diego, USA) according to the manufacturer’s protocol. WGS was conducted using the following steps of the Illumina MiSeq platform: denaturing the libraries; diluting the libraries; preparing the optional PhiX control; loading the libraries onto the reagent cartridge; checking library preparation before inserting into the cartride by KAPA library Quantification Kit Illumina Platform and setting up the sequencing run. The *C. diphtheriae* PW8 complete genome (CP003216.1) was used as a reference sequence.

2.3. Data Analysis

Molecular typing was performed with the MLST approach (Table 1). The profiling of 7 loci was performed, and sequence type determination was conducted online via the MLST global database (https://pubmlst.org/). Since 2022, the database was available on https://bigsdb.pasteur.fr/diphtheria/.

Ethics Statements

The data obtained from archive isolates were exempted from ethical approval as stated by the Health Research Ethics Committee, National Institute of Health Research and Development (HREC-NIHRD): LB.02.01/2/KE216/2017.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Vaccine-preventable diseases Research (Original data) (DDBJ).

CRediT Author Statement

Vivi Setiawaty: Writing – review & editing; Nelly Puspadari: Data curation; Ratih Dian Saraswati: Visualization; Dwi Febriyani: Formal analysis; Tati Febrianti: Data curation; Yuni Rukminiati: Formal analysis; Fauzul Muna: Formal analysis, Data curation; Fitriana Fitriana: Writing – review & editing; Dodi Safari: Writing – review & editing, Data curation; Rahadian Pratama: Writing – review & editing, Data curation; Lisa Andriani Lienggonegoro: Writing – review & editing; Sunarno Sunarno: Conceptualization, Methodology, Writing – original draft.
Acknowledgments

The authors wish to thank the National Institute of Health Research and Development (NIHRD), Indonesia Ministry of Health for funding this research through the 2017 DIPA fund, also thank to Rita Marleta Dewi, Ani Isnawati, the Bacteriology Laboratory Team for data collection and technical assistance and Ageng Wiyatno for data management.

References

[1] N.C. Sharma, A. Efstratiou, I. Mokrousov, A. Mutreja, B. Das, T. Ramamurthy, Diphteria, Nat. Rev. 5 (81) (2019) 1–18, doi:10.1038/s41572-019-0131-y.

[2] R.K. Holmes, Biology and molecular epidemiology of diphtheria toxin and the tox gene, J. Infect. Dis. 181 (Suppl 1) (2000) 156–167, doi:10.1086/315554.

[3] K. Zakikhany, S. Neal, A. Efstratiou, Emergence and molecular characterisation of non-toxigenic tox gene-bearing Corynebacteriumdiphtheriae biovar mitis in the United Kingdom, 2003–2012, Eurosurveillance 19 (22) (2014) 1–8, doi:10.2807/1560-7917.ES2014.19.22.20819.

[4] A. Efstratiou, C. Maple, W.H.O.R.O. for Europe, Laboratory diagnosis of diptheria, Manual for the Laboratory Diagnosis of Diptheria, European Region of World Health Organization, 1994. Available online: https://apps.who.int/iris/handle/10665/108108. Accessed on 1 March 2021.