

Sensitivity of Erythromycin Against Toxigenic Strain of Corynebacterium Diphtheriae

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Research Report

Sensitivity of Erythromycin Against Toxigenic Strain of *Corynebacterium Diphtheriae*

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ABSTRACT

Diphtheria is an acute infection disease caused by *Corynebacterium diphtheriae*. It remains a problem in Indonesia in a recent several years especially in East Java Province, which suffered from an outbreak of diphtheria in 2011. Erythromycin is the second line antibiotics therapy for diphtheria if the patient is allergic to penicillin, also serving as a prophylactic and carrier therapy for contact diphtheria. Erythromycin has been used for diphtheria for a very long time, but there is little recent data on its sensitivity against *C. diphtheriae*. The purpose of this study is to identify whether Erythromycin still has a strong antibacterial activity against *Corynebacterium diphtheriae* by invitro test. This was a descriptive study which observed the sensitivity pattern of erythromycin against *Corynebacterium diphtheriae* using the Epsilon test (etest) as a diffusion technique. Samples used in this study were 30 isolates of toxigenic *Corynebacterium diphtheriae* strain mitis and gravis at the Center for Health Laboratory (BBLK) Surabaya obtained during 2011 until 2014. We retrieved the data based on gender, age, and districts of patients for each of the samples then analyzed them descriptively. In this study, a sensitivity test of 30 toxigenic *Corynebacterium diphtheriae* isolates revealed that 27 (90%) were sensitive to Erythromycin (average Minimum Inhibitory Concentration/ MIC) <0.016 µg/mL and all were strain mitis, while 3 (10%) had intermediate sensitivity with MIC 1 µg/mL (all were strain gravis). No resistance result was found from the sensitivity test. According to the result, we conclude that Erythromycin still has a strong antibacterial activity against *Corynebacterium diphtheriae*.

Keywords: *C. diphtheriae*, Erythromycin, Sensitivity, Epsilon test

ABSTRAK

Difteri merupakan penyakit infeksi akut yang disebabkan oleh bakteri *Corynebacterium diphtheriae*. Difteri masih menjadi masalah di Indonesia dalam beberapa tahun terakhir ini terutama di wilayah Provinsi Jawa Timur yang mengalami Kejadian Luar Biasa (KLB) difteri pada tahun 2011. Eritromisin merupakan antibiotik pilihan kedua bila pasien mengalami alergi terhadap penisilin dalam penanganan difteri, selain itu juga digunakan sebagai terapi karier dan profilaksis kontak difteri. Eritromisin telah digunakan dalam terapi difteri sejak zaman dahulu, namun tidak banyak data publikasi terkini mengenai sensitivitas eritromisin terhadap bakteri *C. diphtheriae*. Tujuan dari penelitian ini adalah untuk mengetahui apakah eritromisin masih memiliki aktivitas antibakteri yang kuat terhadap *Corynebacterium diphtheriae* secara uji invitro. Penelitian ini menggunakan desain deskriptif yang mengamati pola sensitivitas eritromisin terhadap *Corynebacterium diphtheriae* dengan menggunakan teknik difusi epsilon meter (Etest) eritromisin untuk uji sensitivitas. Sampel dari penelitian ini adalah 30 isolat *Corynebacterium diphtheriae* strain mitis dan gravis yang bersifat toksigenik yang terdapat di Balai Besar Laboratorium Kesehatan (BBLK) Surabaya dalam rentang waktu sejak 2011 hingga 2014. Karakteristik sampel yang dihimpun kemudian dikelompokkan berdasarkan jenis kelamin, usia, dan asal daerah pasien dari masing-masing isolata kemudian dianalisis secara deskriptif. Hasil penelitian menunjukkan uji sensitivitas eritromisin terhadap 30 isolat

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Corynebacterium diphtheriae, diantaranya 27 isolat (90%) bersifat sensitif dengan rata-rata Konsentrasi Hambat Minimal (KHM) <0,016 µg/mL dan semuanya merupakan strain mitis, sementara 3 isolat (10 %) memiliki sensitivitas intermediet dengan KHM 1 µg/mL dan semuanya merupakan strain gravis. Tidak ditemukan hasil resisten dalam uji sensitivitas ini. Berdasarkan hasil tersebut, dapat disimpulkan bahwa eritromisin masih memiliki aktivitas antibakteri yang kuat terhadap *Corynebacterium diphtheriae*.

Kata kunci: *C. diphtheriae*, Eritromisin, Sensitivitas, Tes Epsilometer.

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INTRODUCTION

Diphtheria is an acute infection caused by *Corynebacterium diphtheriae* transmitted to humans through respiratory droplets by coughing or sneezing. It can also be transmitted through contaminated clothes after skin diphtheria lesion. The usual symptoms and signs are fever, pain in swallowing, weakness, and a greyish-thick pseudomembrane formed by the growth of bacteria, toxin, necrosis in underlying tissues, and host immunity response. The toxin produced is called diphtheria toxin and is disseminated through the bloodstream, causing systemic infection and organ damage.¹⁻² There are four strains of *Corynebacterium diphtheriae*, namely mitis, gravis, intermedius, and belfanti, which differ from each other according to biological and chemical tests.³

Diphtheria is also one of the world's vaccine-preventable diseases but today still poses a problem in several parts of the world⁴. According to WHO in 2012, Indonesia had the second highest prevalence of diphtheria, with 1192 cases.⁵ Up until October 2012, the number of cases of diphtheria in East Java was as many as 710 and Situbondo district had the highest prevalence, with 113 cases and 7 deaths from it.⁶

The first-line antibiotic used for diphtheria is penicillin due to its bactericidal action compared to Erythromycin as a bacteriostatic^{7,8}. Unfortunately, in Indonesia penicillin is only available in the form of an injection that has to be given intramuscularly, which is uncomfortable. Oral Erythromycin is an alternative antibiotic for those who are hypersensitive to penicillin,

and as a prophylactic treatment. The secondary use of Erythromycin is for the eradication of *Corynebacterium diphtheriae*.^{9,10} Erythromycin has been widely used in daily treatment for other respiratory infections for a very long time, raising questions about its sensitivity against *C. diphtheria*. There are very few recent studies or data about the sensitivity of Erythromycin against *Corynebacterium diphtheriae*, especially in East Java Province, Indonesia. Therefore, we conduct a study to identify whether Erythromycin still has an antibacterial activity against *Corynebacterium diphtheriae*.

MATERIALS AND METHODS

This is a descriptive study to observe the sensitivity pattern of Erythromycin against *Corynebacterium diphtheriae*. The samples are retrieved from 216 *C. diphtheria* isolates collected during diphtheria outbreaks in East Java Province between 2011 until 2014, stored at the Center for Health Laboratory (BBLK), Surabaya, as the national referral laboratory for diphtheria in Indonesia. The isolates came from several districts and cities in East Java Province, and consist of *C. diphtheria* strains mitis and gravis. We used the stratified sampling method to determine 30 isolates of *Corynebacterium diphtheriae* as the sample size. We divided the total population (216 isolates) into groups based on their district/city of origin, then we proportionally counted the number of samples needed from each district group based on their incidence rate: Bangkalan 18 (60%), Jember 5 (16.7%), Bondowoso 4 (13.3%),

Banyuwangi 1 (3.3%), Bojonegoro 1 (3.3%), and Tuban 1 (3.3%). Isolates from each district/group were then simply chosen randomly. The inclusion criterion was toxigenic isolates determined by Elek tests,¹¹ while the exclusion criterion was isolates that did not grow in agar medium and showed negative in nitrate and glucose tests. The sensitivity of Erythromycin was tested by the Epsilon test (Etest) as a diffusion technique.¹² The result was interpreted based on the Erythromycin MIC in accordance with the Clinical Laboratory Standard and Institute (CLSI), where MIC \leq 0.5 $\mu\text{g/ml}$ is sensitive, MIC = 1 $\mu\text{g/ml}$ is intermediate, and MIC \geq 2 $\mu\text{g/ml}$ is resistant.¹³ The number of isolates showing sensitive, intermediate, or resistant results were explained descriptively. The study was approved by the Medical Research Ethics Commission of the Faculty of Medicine, Universitas Airlangga No:191/EC/KEPK/FKUA/2014.

RESULTS AND DISCUSSION

The characteristics of patients with positive *Corynebacterium diphtheriae* cultures used in this study are shown in Table 1. From the 30 isolates tested, 53.3% were obtained from patients aged \geq 15 years and the highest prevalence came from Bangkalan district (60%). This was different from the data based on East Java Health Office in 2012, which showed that diphtheria cases in the < 15 years age group were more prevalent and that most cases of diphtheria came from Situbondo district.⁶

Based on the sex distribution, 66.7% of isolates were obtained from female patients. A study by Volzke in Germany showed that women without toxoid immunization had four times the risk of suffering from diphtheria compared to non-immunized men.¹⁴ The study by Nath et al. in India showed a different result, however, and from 60 cases of diphtheria reported, males were affected more than females, with figures of 53.33% and 46.67% respectively.¹ Meanwhile, the majority *C. diphtheriae* strain found was *C. diphtheriae* mitis (90%), while strain gravis accounted for 10%, and neither the intermedius nor the belfanti strain was found.

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Table 1. Characteristics of Patients with Positive *Corynebacterium Diphtheriae* Culture in this Study

No.	Category	Frequency	%
1	Age (Year)		
	< 15	14	46.7
	\geq 15	16	53.3
2	Sex		
	Male	10	33.3
	Female	20	66.7
3	Origin		
	Bangkalan	18	60
	Jember	5	16.7
	Bondowoso	4	13.3
	Banyuwangi	1	3.3
	Bojonegoro	1	3.3
	Tuban	1	3.3
4	Strain		
	Mitis	27	90
	Gravis	3	10
5	Sensitivity of Erythromycin		
	Sensitive	27	90
	Intermediate	3	10
	Resistant	0	0

The results of the present study are shown in Table 2, which makes clear the significant finding of 90% sensitive results with an average MIC < 0.016 $\mu\text{g/mL}$ (MIC \leq 0.5 $\mu\text{g/ml}$ was sensitive) and 10% intermediate results (MIC 1 $\mu\text{g/ml}$ was intermediate). No resistance was found (MIC \geq 2 $\mu\text{g/ml}$). Clinically, we should increase the dose therapy of Erythromycin for the management of diphtheria based on the invitro intermediate results in order to eradicate *Corynebacterium diphtheriae*. Although the strain mitis has much greater prevalence than gravis (90% mitis and 10% gravis), it can be treated by a normal dose of Erythromycin based on its sensitivity result to *Corynebacterium diphtheriae* (strain mitis 100% sensitive, gravis 100% intermediate).

Few studies have been conducted recently on the sensitivity of antibiotics against *Corynebacterium diphtheriae* and, of these, several studies are outdated because the number of cases of diphtheria has declined significantly in recent years due to good immunization coverage and surveillance

Table 2. Sensitivity of Erythromycin against *Corynebacterium diphtheriae* by Epsilon Meter Test (Etest)

No	Gender	Age	Origin	Strain	MIC ($\mu\text{g/mL}$)	Interpretation
1	Male	40	Bangkalan	Mitis	< 0.016	Sensitive
2	Male	5	Bangkalan	Mitis	< 0.016	Sensitive
3	Female	4	Bangkalan	Mitis	0.016	Sensitive
4	Male	4	Bangkalan	Mitis	< 0.016	Sensitive
5	Female	20	Bangkalan	Mitis	0.016	Sensitive
6	Female	6	Bangkalan	Mitis	0.016	Sensitive
7	Female	23	Bangkalan	Mitis	< 0.016	Sensitive
8	Male	6	Bangkalan	Mitis	0.016	Sensitive
9	Female	12	Bangkalan	Mitis	< 0.016	Sensitive
10	Male	6	Bangkalan	Mitis	< 0.016	Sensitive
11	Female	37	Bangkalan	Mitis	< 0.016	Sensitive
12	Female	17	Bangkalan	Mitis	< 0.016	Sensitive
13	Female	7	Bangkalan	Mitis	< 0.016	Sensitive
14	Female	25	Bangkalan	Mitis	0.016	Sensitive
15	Male	15	Bangkalan	Mitis	0.016	Sensitive
16	Male	18	Bangkalan	Mitis	< 0.016	Sensitive
17	Female	36	Bangkalan	Mitis	< 0.016	Sensitive
18	Female	6	Bangkalan	Mitis	< 0.016	Sensitive
19	Female	18	Banyuwangi	Mitis	< 0.016	Sensitive
20	Female	13	Bojonegoro	Mitis	< 0.016	Sensitive
21	Female	13	Tuban	Mitis	< 0.016	Sensitive
22	Female	12	Bondowoso	Mitis	< 0.016	Sensitive
23	Female	16	Bondowoso	Mitis	< 0.016	Sensitive
24	Female	29	Bondowoso	Gravis	1	Intermediate
25	Male	9	Bondowoso	Mitis	< 0.016	Sensitive
26	Female	20	Jember	Mitis	< 0.016	Sensitive
27	Female	52	Jember	Mitis	< 0.016	Sensitive
28	Male	17	Jember	Mitis	< 0.016	Sensitive
29	Male	11	Jember	Gravis	1	Intermediate
30	Female	16	Jember	Gravis	1	Intermediate

systems, especially in well developed countries. The result of the current study was similar to several previous studies. Gordon (1970) in Texas, USA, used the dilution technique of sensitivity against *Corynebacterium diphtheriae* and showed that all of the 14 toxigenic isolates were sensitive, with MIC 0.01 $\mu\text{g/ml}$.¹⁶ McLaughlin (1971) in Atlanta, USA, used Erythromycin 15 μg in a disk diffusion technique for a sensitivity test against *Corynebacterium diphtheriae* and showed that 136 isolates between 1969–1970 were sensitive, with a mean MIC of approximately 40 mm (sensitive ≥ 23 mm).¹⁷ The study by Rockhill et al. in Jakarta, Indonesia (1980), also using the disk diffusion technique with Erythromycin 15

μg , showed that 133 isolates were all sensitive to Erythromycin.¹⁸ Engler et al. (2000) in England also found that 405 of 410 isolates were sensitive, with MIC 0.026 $\mu\text{g/ml}$, and 5 others were resistant with MIC of 2–4 $\mu\text{g/ml}$.¹⁹ Another study from Barraud et al. in France using the Etest method showed the susceptibility of many antibiotics including Erythromycin against 46 isolates of *Corynebacterium diphtheriae* biovar mitis in the period from 1993 to 2010.²⁰ A study in Russia by Chagina et al. using the Etest method showed that of 664 isolates between 1987–2013, most of them turned out to be sensitive to all antibacterial preparation, although 0.4–0.6% were intermediate and 4–4.4% were resistant to macrolide.²¹

CONCLUSION

Erythromycin still has a strong antibacterial activity against *Corynebacterium diphtheriae* (90% sensitive, 10% intermediate). The use of Erythromycin for the management of diphtheria, especially for those who have penicillin allergy, or as a prophylactic treatment is recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for this research.

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