

The 4th International Conference on Medical Laboratory Technology (ICoMLT)

**Disk Diffusion Method Of Antibiotic Susceptibility Testing With Erythromycin On
*Corynebacterium diphtheriae***

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ABSTRACT

Corynebacterium diphtheriae is the bacterium that causes diphtheria. The high mortality of diphtheria cases is mainly due to delays in diagnosis and therapy. One of the measures to prevent diphtheria is by giving prophylaxis to cases, contacts and carriers. The recommended antibiotics of choice are penicillin and erythromycin. Bacterial susceptibility tests to antibiotics need to be carried out continuously to determine the development of bacterial resistance. Based on this, it is felt that the diffusion disk method antibiotic sensitivity test needs to be developed to support culture examination and antibiotic sensitivity testing for diphtheria at BBLKM Surabaya. This method is used as an alternative to the standard dilution method and is expected to have good suitability. The purpose of the study was to determine the results of the diffusion disk method sensitivity test with erythromycin antibiotics against *Corynebacterium diphtheriae* bacteria that are still sensitive or resistant. This study is an observational descriptive study conducted on 33 positive isolates of *Corynebacterium diphtheriae* in 2023 at BBLKM Surabaya. The research was conducted in the clinical microbiology laboratory of BBLKM Surabaya in May 2024. The results showed sensitive results with a diameter of more than 24 mm in all isolates tested with details of the percentage of samples with an inhibition zone of 25 mm as much as 6%, samples with an inhibition zone of 36 mm as much as 3%, samples with an inhibition zone of 37 mm as much as 19%, samples with an inhibition zone of 25 mm as much as 6%.

Keywords: *Corynebacterium diphtheriae*; Susceptibility testing; Erythromycin; Disk diffusion

BACKGROUND

Corynebacterium diphtheriae attacks the respiratory tract and causes symptoms such as sore throat, fever and swollen lymph nodes. The disease is very dangerous and can cause death if not treated immediately (Hidayathillah et al, 2023). The complexity of diphtheria disease and the threat of spreading cases in several regions in Indonesia indicate the importance of identifying the bacteria that cause diphtheria in Indonesia (Sunarno et al, 2015). One of the measures to overcome diphtheria is to provide prophylaxis to cases, contacts and carriers, namely by giving antibiotics to stop the production of bacterial toxins and prevent transmission in

the surrounding community. The recommended antibiotic programs of choice are penicillin and erythromycin (Ministry of Health, 2017). Despite the variety of methods, in-vitro antimicrobial susceptibility testing has the common goal of providing a reliable predictor of how an organism might respond to antimicrobial therapy in an infected host or to assess for surveillance purposes whether resistance development has occurred (OIE, 2019). In the liquid dilution MIC method, most antimicrobial test panels are commercially prepared, this method is less flexible than agar dilution or disc diffusion in adjusting to the changing needs of the

surveillance/monitoring program. As for the agar dilution MIC method, if it is not automated it is very laborious and requires large economic costs and technical resources (OIE, 2019).

This study aims to determine the sensitive and resistant values of the disk diffusion method sensitivity test with erythromycin antibiotics against *Corynebacterium diphtheriae* bacteria. Based on this, it is felt that the antibiotic sensitivity test of the disk diffusion method needs to be developed to support culture examination and antibiotic sensitivity testing of diphtheria in BBLKM Surabaya. This method is used as an alternative to the standard liquid agar dilution method and is expected to have good suitability.

RESEARCH METHODS

This study uses an observational descriptive design using secondary data in the form of identification results of positive isolates of *Corynebacterium diphtheriae* and primary data in the form of erythromycin antibiotic sensitivity test results against *Corynebacterium diphtheriae* Disk Diffusion method. The samples of this study were positive *Corynebacterium diphtheriae* isolates from patients in 2023 at BBLKM Surabaya as many as 33 samples. Sampling was done by Simple Random Sampling. The research was conducted at the Clinical Microbiology Laboratory of the Surabaya Public Health Laboratory Center in May 2024.

RESULTS AND DISCUSSION

After phenotypic tests to identify the species of *Corynebacterium diphtheriae*, antibiotic sensitivity tests were then carried out using the disk diffusion method with the antibiotic erythromycin. The antibiotic sensitivity test for *Corynebacterium diphtheriae* bacteria uses Mueller Hinton media + 5% sheep blood which is spotted with 0.5 Mac Farland bacterial suspension then erythromycin disk is attached and incubated at 35°C for 1x24 hours. After incubation, the inhibition zone formed was

measured using a caliper and written in millimeters (mm) then interpreted according to EUCAST guidelines. The results of the disk diffusion method sensitivity test with erythromycin antibiotics against *Corynebacterium diphtheriae* bacteria in 33 samples showed sensitive results with a percentage of 100% and resistant results of 0%. The interpretation of the results is in accordance with EUCAST 2024 guidelines where the results of the erythromycin antibiotic sensitivity test against *Corynebacterium diphtheriae* species with the disk diffusion method are said to be sensitive if the diameter of the inhibition zone is ≥ 24 mm and resistant if the diameter of the inhibition zone is < 24 mm. Of the 33 samples, all had inhibition zones > 24 mm.

Diphtheria is a life-threatening disease, so a clinical diagnosis must be made immediately. Once a suspected clinical diagnosis is made, the patient should be isolated immediately to reduce the risk of spreading the toxigenic strain to other susceptible people and receive immediate antitoxin and antibiotic therapy. Antibiotics such as penicillin and erythromycin can be given parenterally, until the patient can swallow well. (Goering et al, 2019)

This study was conducted with the aim of knowing whether the antibiotic erythromycin as the government's first choice antibiotic used for the treatment of diphtheria is still sensitive or has decreased its effectiveness against diphtheria germs.

For a long time, erythromycin has been the prescribed treatment for diphtheria; nevertheless, numerous investigations have shown that this drug's susceptibility has decreased. The gastrointestinal system is another issue with this medication. In the clinical setting conducted by dr. Dominicus Husada in his research, only a few people were able to complete a 7-day course of erythromycin. In the 1980s, erythromycin side effects on the heart were documented. Unfortunately, information about carriers and patients with

diphtheria has not been gathered and reported in Indonesia. The only type of erythromycin that is sold in this nation is oral.

Erythromycin is a good example of a macrolide antibiotic. This antibiotic is produced by *Streptomyces erythreus*. Erythromycin selectively inhibits bacterial growth by binding to bacterial ribosomes and reversibly inhibiting protein synthesis. Reversible action on bacterial ribosomes indicates that its antimicrobial effect is bacteriostatic. (Kuswandi, 2023).

CONCLUSION

From the results of this study it can be concluded that:

The results of the disc diffusion method sensitivity test with erythromycin antibiotics against *Corynebacterium diphtheriae* in 33 patient isolates in 2023 all had an inhibition zone diameter > 24 mm which showed 100% sensitive results and 0% resistant results in accordance with EUCAST 2024 guidelines. This shows that erythromycin antibiotics still have good effectiveness to be used as the antibiotic of choice for the treatment of diphtheria. The results of this study can be used as support for culture examination and antibiotic sensitivity testing of *Corynebacterium diphtheriae* at BBLKM Surabaya

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