

EVALUATION OF ANTIBIOTICS DOXYCYCLINE: POTENTIAL TEST AND COMPARATIVE TEST OF ACTIVITY IN ANTIBIOTIC RESISTANCE CASES OF ARIS AT TASIKMALAYA CITY HEALTH CENTER, INDONESIA

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ABSTRACT

Objective: Acute respiratory tract infection (ARIs) is a disease attacking one or more parts of the respiratory tract. The treatments of ARIs, people tend to give some antibiotics. The purpose of this study is to obtain data from the quality of antibiotics doxycyclin used viewed from the parameters of potential test and comparative test of activity against clinical isolates of patients with ARIs at Purbaratu Tasikmalaya City Health Center. This data is useful for evaluating the effectiveness of antibiotic use and determining government policy in the handling of cases of resistance. **Methods:** The research methods include the rejuvenation of clinical isolates, potential test, and comparative test of activity antibiotic. **Results:** The antibiotic potential test result is 86.8%, and the comparative test of antibiotic activity is 1: 1.03. **Conclusions:** The value of the antibiotic test of doxycycline has decreased based on the requirements listed on Pharmacopoeia Indonesia. Comparative tests of antibiotic activity indicate that the doxycycline samples used in community health centers have activities equivalent to antibiotic standards.

KEYWORDS: ARIs, antibiotic, doxycycline, potential test, comparative test.

INTRODUCTION

Acute respiratory infection is a serious infection that prevents normal breathing function. It usually begins as a viral infection in the nose, trachea (windpipe), or lungs. If the infection is not treated, it can spread to the entire respiratory system. Acute respiratory infection prevents the body from getting oxygen and can result in death. Person suffering from this condition needs medical assistance immediately. Also, acute respiratory infections are infectious, which means they can spread from one person to another. The disease is quite widespread. It is particularly dangerous for children, older adults, and people with immune system disorders.^[1]

ARIs cases in Indonesia cause deaths in infants by 28%. Based on the results of the study that the prevalence of ARI in Indonesia is 25.5% (range: 17.5% - 41.4%) with 16 provinces of which have prevalence above the national rate. Based on the result of basic health research of West Java province, the prevalence of ARI in Tasikmalaya City gives the real number to national prevalence number.^[2]

The main cause of ARIs is the ineffective use of antibiotics. Compliance with the use of patient antibiotics and the accuracy of physician prescribing of

an infection symptom play a dominant role in reducing the occurrence of ARIs. The resistance of an antibiotic may also cause ARIs treatment to be inaccurate and efficient. The potential value and antibiotic activity is a quality parameter of an antibiotic in the successful treatment of an infectious disease. In this study, we studied the phenomenon of resistance occurring in the use of antibiotics seen from the antibiotic quality parameters of he potential value and antibiotic activity.

RESEARCH METHODS

Tools and materials

In this study the tools used in this study were autoclave (Hirayama), micropipet volume 5-1000 μ L (Eppendorf), tip micropipet, incubator (Sakura IF-4), sliding range, spritus burner, Ose, spatel, analytical balance, whatman no. 41 paper, glass vials, and glass tools commonly used in the Microbiology.

The materials used in this study consisted of several samples of antibiotics used in health center, standard tertiary antibiotics from PT. Sanbe Farma, antibiotic solvents, bacterial growth medium, chemicals and biochemical test materials.

RESEARCH METHODS

The steps of this study were to rejuvenate clinical isolates, determine the potency of doxycycline antibiotics, Examine the value of comparative activity of doxycycline antibiotics against the doxycycline standard, which is used in public health centers in the city of Tasikmalaya, Indonesia.

Rejuvenation of Clinical Isolates. Clinical isolate samples obtained from previous studies were rejuvenated by replanting bacteria into new media similar to the previous medium, MHA. Rejuvenation is done by taking bacteria from the previous media by using Ose then scratched into new media. After that it was incubated for 18-24 hours at 37°C. After that, we observed the colony morphology of different colors, colonies, and morphological and haemolytic properties.

Preparation of a Doxycycline Antibiotic Solution. The doxycycline antibiotic solution is prepared by the powder of antibiotic removed from the capsule then weighed the equivalent of 10 mg of the active ingredient then dissolved in 0.1 N HCl until dissolved. Then added distilled water to 100 mL.^[3]

Preparation of Bacterial Suspension Test. Preparation of bacterial suspension test is done by inoculating bacterial colony into sterile physiological NaCl solution. Turbidity of bacterial suspension should be equivalent to the standard turbidity of 0.5 Mc Farland solution.^[4]

Testing of the potential of doxycycline antibiotics using agar diffusion. Suspension of prepared bacteria, poured into sterile petri dish as much as 20 µl. Then medium MHA temperature 45°C poured as much as 20 mL into the petri dish. The bacterial suspension and the media in the petri dish are then homogenized and allowed to solidify. The base of this petri dish is then patterned into 6 areas, then perforated using perforator aseptically. Each of the holes is then filled with a sample and standard antibiotic solution according to the variation of the concentration previously selected. In addition to the test media, also made a positive control containing media and suspension of test bacteria, and negative control consisting of MHA media. Thereafter, the test medium and incubation control for 18-24 hours at 37°C. Determination of antibiotic potency using 3 dose calculation done by formula:

$$I = \log \frac{DT}{DM} = \log \frac{DM}{DR}$$

$$E = \frac{1}{4} \times [(St - Sr)] + [(Bt - Br)]$$

$$b = \frac{E}{I}$$

Comparative Test of Activity Antibiotic Used In Public Health Center To Standard Doxycycline Antibiotics.

Suspension of prepared bacteria is poured in sterile petri dishes. After that, MHA media with a temperature of 40-45°C poured into a petri dish as much as 45 mL then homogenized and allowed to harden. Petri dishes containing inoculum solution are then divided into 8 parts and each perforated using perforator aseptically. Each of these holes is then filled with standard antibiotic samples and solutions according to predetermined concentration variations. In addition to test media, also made positive control containing media and suspension of test bacteria, as well as negative control consisting of MHA media. Thereafter, the test and control media were incubated for 18-24 hours at 37°C.

Samples and standards were measured at each inhibitory diameter and then made standard curve with the equation of line $y = ax + b$ where y as diameter and x as log concentration. After obtaining linear line equation, it is determined that one inhibitory diameter entered in the test range is then plotted in the line equation and determined the sample and raw concentration of each isolate.^[5] The value of the comparative test activity can also be determined by the equation.^[6]

$$\text{Comparative Test Activity} = \frac{\text{Concentration of test substances}}{\text{Comparative antibiotic concentration}}$$

RESULTS AND DISCUSSION

Results of Antibiotic Potential Test Determination

An antibiotic potential test was performed on antibiotic samples used in Purbaratu, Public Health center with standard antibiotic doxycycline. This test was conducted to overcome any doubts about the possible loss of activity or antibiotic potential for the effects of inhibitory power on microbes. The principle of determining the antibiotic potential in the drug preparation is to compare the dosage of the test preparation solution to the dose of the reference standard solution resulting in the degree of inhibition on the test microorganism.^[7]

The potential test used is with 3 + 3 pattern where 3 variations of sample dose and 3 variations of comparator dose are used. The dose used was 2 µg / mL as a high dose, 1 µg / mL as a median dose and 0.5 µg / mL as a low dose. Potential testing is done three repetitions. The results of potential calculations with a three-dose pattern obtained for 86.8%. These results are then compared with the standards contained in the Indonesian Pharmacopoeia between 88% -98%.^[3] The results can be seen that the antibiotic potential of doxycycline samples used by Purbaratu Health Center Tasikmalaya City has decreased. The decrease in antibiotic potency can be caused by several factors, such as near expiration time or antibiotic storage that is not performed in accordance with the recommended storage conditions.

Results of Comparison Test

This antibiotic comparison test was performed on bacteria from the most sensitive clinical isolates, the

bacteria with the greatest inhibitory zone. This is done to determine the level of sensitivity of antibiotics inhibiting the activity of bacteria that cause ARIs so that it can be used as a guide in the proper treatment for ARI disease. The dosage of the test used is determined based on the results of the previous optimization. The dose used after optimization is 0.8 µg / mL; 0.4 µg / mL; 0.2 µg / mL and 0.1 µg / mL. The drag zone diameter of the test result of the appeal value has been seen in the table below.

Table 1: Diameter of inhibition on comparative test.

Antibiotic	Concentration of Antibiotics			
	0.8 µg/mL	0.4 µg/mL	0.2 µg/mL	0.1 µg/mL
Standar Doxycycline	18.87	16.24	14.44	12.63
Sample Doxycycline	18.57	16.30	14.34	12.47

The measurement of the comparative test activity is obtained from the curve making between the log of concentration to the inhibit zone of the standard and the sample. The linear line equation for the standard is $y = 6.82x + 19.3$ with $R^2 = 0.994$. The equation curve for standard can be seen in Figures 1.

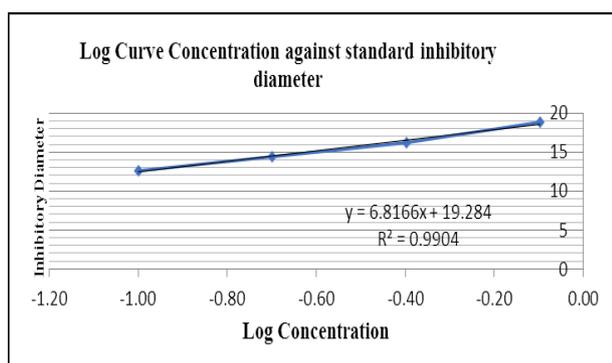


Figure 1: Log curve Concentration against standard inhibitory diameter.

The linear line equation for the sample is $y = 6.73x + 19.11$ with $R^2 = 0.9979$.

The equation curve for standard can be seen in Figures 2.

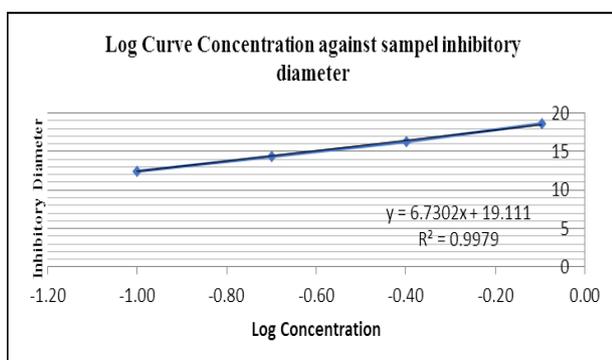


Figure 2: Log Curve Concentration against sampel inhibitory diameter.

After obtaining the equation, one of the concentration logs of the standard is substituted as x into the standard equation, so that the value of y is obtained as the drag zone diameter. The value of y is then substituted into the equation of the sample line so that the value x is obtained. The graph of result of the comparative test activity value between standard and sample can be seen in Figure 3.

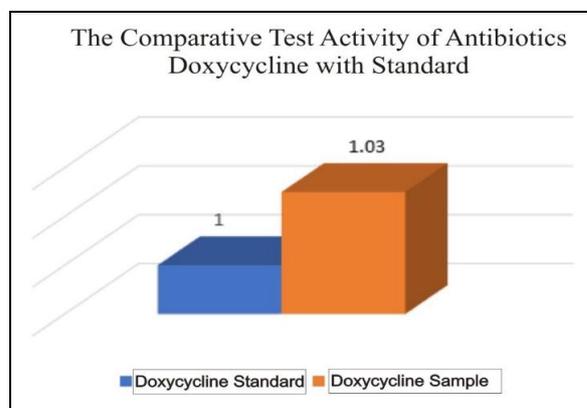


Figure 3: The comparative test activity of antibiotics doxycycline with standard.

The test results obtained comparison between the standard with the sample is 1: 1.03. This suggests that the standard doxycycline antibiotics used have the ability to kill sensitive clinical isolate bacteria which are slightly better than the antibiotic samples. Based on these results it can be seen that the concentrations required by sample antibiotics to produce an equivalent effect do not vary much with the standard antibiotic comparison.

CONCLUSIONS

This research can be concluded that doxycycline antibiotics used in Public Health Center, Purbaratu in Tasikmalaya has antibiotic potency 86.8%. This value is already declining, because it is not in accordance with the requirements on the Indonesian Pharmacopoeia is 88-98%. The result of comparative activity test is 1: 1.03.

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