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EVALUATION OF CEFIXIME ANTIBIOTIC RESISTANCE: RELATION OF ANTIBIOTIC QUALITY PARAMETERS TO CASES OF RESISTANCE OCCURRING IN ARIS DISEASE IN TASIKMALAYA CITY HEALTH CENTER.

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ABSTRACT

Objective. Cefixime is an antibiotic that usually used in treatment of acute respiratory infections (ARIs). One of the factors that support the occurrence of cases of antibiotic resistance is the antibiotic quality parameters of the potential and levels of antibiotics used. This study aims to determine the relationship between quality parameters of cefexime antibiotics used in public health centers in Tasikmalaya city with cases of cefexime resistance occurring in the area. **Methods.** This research method includes the determination of antibiotics potential with 3+3 pattern and the comparative value of the activity using agar diffusion method perforation techniques and antibiotic assay with UV-Visible spectrophotometry method. **Results**. The research shows the antibiotic potential of cefixime is 95.86%. The validation method demonstrate that the method used fulfill the requirements of standard curve with concentrations in the range of 6-14 mg/L with a correlation coefficient of 0.995. The method used demonstrated accuracy with an average recovery percent of 100.08 ± 2.39% and a precision of 0.06%. LOD value of the method used is 0.1236 mg/L while the LOQ value is 0.4121 mg/L. The Results of analysis showed levels of cefixime is 100.71%.

KEYWORDS: ARIs, antibiotics, cefixime, potency, antibiotic assay, validation method.

INTRODUCTION

Acute respiratory infection (ARIs) is an acute infection of any part of the respiratory tract and associated structures including sinus paranasal, middle ear and pleural cavity (1). While the acute sense is an infection that lasts up to 14 days. ARIs is a major cause of infectious morbidity and mortality in the world. Nearly four million people worldwide die from ARIs each year. The mortality rate is very high in infants, children, and the elderly, especially in countries with low and medium income per capita (2). ARIs cases constitute 50% of all illnesses in children under five years of age, and 30% in children aged 5-12 years. Research by The Board on Science and Technology for International Develeopment shows that the incidence of ARI in children under 5 years reaches 12.7 to 16.8 episodes per 100 child-weeks (child-weeks) (3).

The results of calculations in Indonesian hospitals found that 40% to 70% of patients with ARI treated to the hospital are children. Patients with respiratory infection (ISPA) treated at puskesmas were 40-60% and 15-30% of patients had access to outpatient and inpatient (4).

ARIs can be caused by bacteria, viruses, and rickets. Bacteria that cause ISPA include Streptococcus genus, Staphylococcus, Pneumococcus, Hemophilus, Bordetella, and Corynebacterium. antibiotics that are often used in the treatment of ARD are cephalosporin (cefotaxim, cefadroxil, ceftriaxon and cefixime) (5). Cefixime is an oral third-generation cephalosporin antibiotic, has antimicrobial activity against both positive and negative Gram bacteria including Enterobacteriacea. On oral administration, nearly 50% immediately reach bactericidal concentrations and penetrate tissues well (6).

Some factors that support the occurrence of resistance are too low doses and low potential (7). This study aims to determine the relationship between antibiotic quality parameters with cases of resistance occurring in Tasikmalaya city health center.

MATERIALS AND METHODS

Tools

The tools used in this study were autoclave (Hirayama), micropipette with a volume range of 10-100 µL (Eppendrof), incubator (Sakura IF-4), sliding range, spectrophotometer (SPECORD 200-222U203), paper whatman no.41 , ultrasonic bath (NEY 1510), and glass tools commonly used in the Laboratory of Microbiology and Chemical Analysis.

MATERIALS

The materials used in this study consisted of antibiotic samples from Tasikmalaya City Health Center, standard cefixime antibiotics from PT Meprofarm, antibiotic solvents, test bacteria, media for bacterial growth (Oxoid), motility test media, biochemical test materials, distilled water and methanol pro analysis (JT Baker).

METHODS

Determination of Antibiotic Potential Test

a. Preparation of Bacterial Suspension

The bacteria used for testing the antibiotic potential of cefixime is Staphylococcus aureus ATCC 25923. Preparation of bacterial suspension is done by scratching one Ose pure strain bacteria then inserted into sterile physiological NaCl solution and measured turbidity up to 0.5 Mc Farland.

b. Preparing Antibiotic Solution

The cefixime antibiotic solution was prepared by cefixime removed from the capsule and weighed equivalent to 100mg of the active ingredient, then dissolved with a solution of D1 to 100mL. Solution D1 was prepared by dissolving as much as 2.0g of aqueous potassium phosphate and 8.0 g of potassium phosphate monobase dissolved in 1L of distilled water. The pH is adjusted to 6.0±0.05 with 18 N phosphoric acid or 10 N N potassium hydroxide. The cefixime solution is sterilized using an autoclave at 121 ° C for 15 minutes. Then dilution with sterile distilled water to achieve the concentration of 15 mg/mL, 10 mg/mL, and 5 mg/mL (8).

c. Testing Potential Antibiotics

Testing is done by pouring bacterial suspension into sterile petri dish. Then poured MHA media (40-45°C) as much as 20 mL then homogenized and allowed to freeze. The petri dish containing the inoculum solution is divided into 6 parts and each part is made a hole (reservoir) using perforator aseptically. Each hole is filled with a sample and standard antibiotic solution according to the concentration variation. Subsequently incubation for 18-24 hours at 37 ° C.

d. Determination of Potential Antibiotics 3 Doses

After incubation, the inhibitory diameter of each reservoir is determined. Determination of antibiotic potency using 3 doses is done by using the following formula:

E = 1/4 x [(St-Sr)] + [(Bt-Br)]

b = E / $\log^2$

F = 1/3 x [(St + Sm + Sr)] - [9Bt + Bm + Br]]

M = F / b

Potential = antilog M x 100% (8).

Antibiotic Assay

1. Determination of Maximum Wavelength

The maximum wavelength was determined by measuring a standard solution of 10 µg / mL cefixim prepared from a cefixime solution of 100 µg/mL and then observed its maximum absorbance using a UV-V is spectrophotometer.

2. Determination of Standard Curve

Measurement of standard cefixime solution with 6 µg/mL concentration; 8 µg/mL; 10 µg/mL; 12 µg/mL; and 14 µg/mL at maximum wavelength. The standard curve shows the relationship between concentration and absorbance so that the equation of the line in the form y = ax + b.

3. Analysis Validation Method

a. Linearity Test

The raw curve obtained, will get the value of the correlation coefficient (R^2) which states the value of linearity. The linearity parameter is expressed in the correlation coefficient (R^2) whose value is more than 0.99 at a minimum of five concentration variation points (9).

b. Determination of Limit of Detection (LOD), Limit of Quantification (LOQ)

The standard curve has been obtained, calculated the number of detectable smallest analyte (LOD) with the equation:

LOD = [3 (Sx / y)] / slope

And calculated also the smallest quantity of analytes that can still meet the criteria with the equation:

LOQ = [10 (Sx / y)] / slope.

c. Precision Test

Precision is expressed as the value of the relative standard deviation or coefficient of variation. acceptable parameters of precision ie < 2% (10).

d. Accuracy Test

Accuracy is obtained through% recovery (% recovery) with the equation:

% recovery = (analysis result) / (actual content) x 100%

the required range of the% recovery values is 80 - 110% (10).

4. Quantitative Analysis of Sample

The sample was prepared, then weighed as much as 5 mg then dissolved in a 50 mL measuring flask with methanol and obtained a sample solution of 100 µg / mL. Samples were diluted to obtain 20 µg / mL sample by taking as much as 5 mL and dissolved in 25 mL measuring flask with methanol. Absorbance was observed at its maximum wavelength using a UV-Vis spectrophotometer.

RESULTS AND DISCUSSION

1. Antibiotic Potential Test

An antibiotic potency test was performed by comparing antibiotic samples which are used in Tasikmalaya City Health Center, with a standard cefixime antibiotic. Potential test was used 3 dose variations for the sample and 3 dose variations for the reference standard (pattern 3 + 3). The dose was determined by optimization first and obtained high dose of 15 µg / mL, medium dose of 10 µg / mL, and low dose 5 µg / mL. The inhibitory diameter resulting can be seen in Table 1.

Table 1. Potential Test Results.

Average Inhibitory Diameter (mm)					
SdH	**SdM**	**SdL**	**SpH**	**SpM**	**SpL**
22,55	20,15	15,11	22,34	19,68	15,12

SdT : Standard High Dose SpH : Sample High Dose

SdM : Standard Medium Dose SpM : Sample Medium Dose

SdL : Stdandard Low Dose SpL : Sample Low Dose

The cefixime potential test result is 95.86%. These results show that cefixime antibiotics meet the requirements set at the Indonesian Pharmacopoeia 95% - 105% (8).

2. Antibiotic Assay

Determination of Maximum Wavelength

In this study, the maximum wavelength was obtained at 291nm. This maximum wavelength is slightly larger than the literature of 289nm (11). This wavelength difference is due to

differences in methanol solvent specifications used. The spectra of standard cefixime standard wavelength with UV-Vis spectrophotometer can be seen in Figure 1.

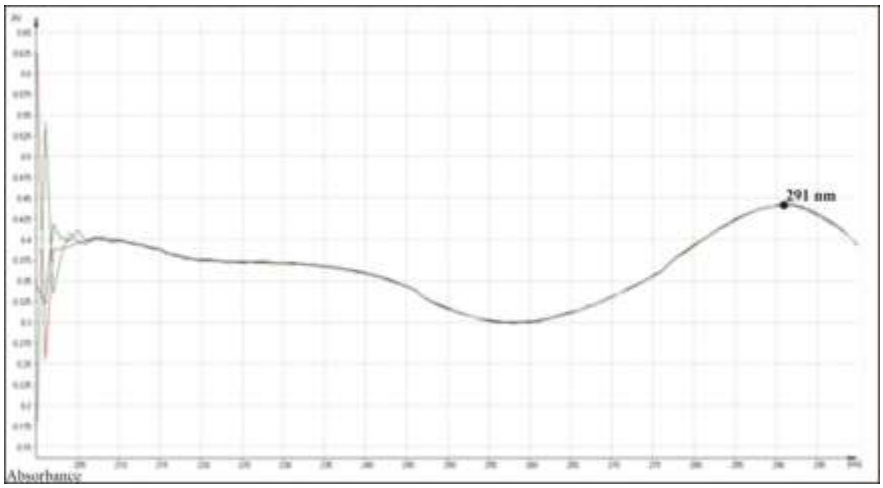

Figure 1. Spectrum maximum wavelength standard cefixim 10 ppm (291nm).

Determination of Standard Curve

The standard curve is a plot between absorbance and concentration. Based on regression obtained regression coefficient (r) (12). Linear regression equation was obtained y = 0,0517x-0,0249 with r^2 = 0,995. The result of standard curve can be seen in Figure 2.

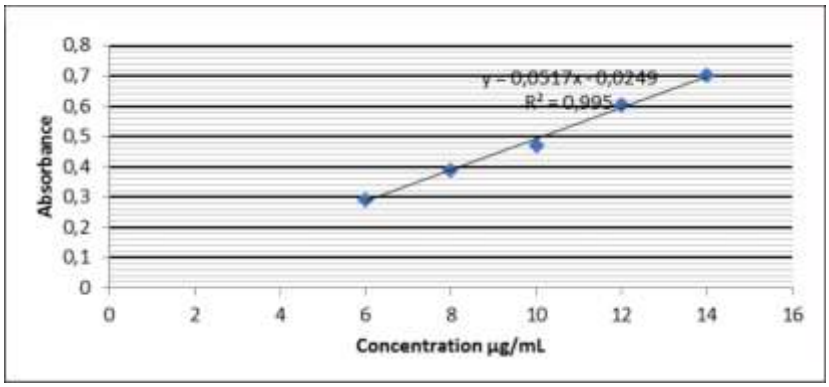

Figure 2. Standard curve of cefexime standar solution.

A value of R^2 close to 1 proves that the regression equation is linear and the small standard deviation indicates high accuracy.

Analysis Validation Method

a. Linearity Test

The linearity parameter is expressed in the correlation coefficient (r2) whose value is more than 0.99 at a minimum of five concentration variation points (ICH, 1994). In this research,

r2 = 0.995. The value of correlation coefficient which is very close to 1 represents a very linear relationship between the sample concentration with the detector response which means the equation of the line can be used for sample measurement (10).

b. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values can be calculated through the linear regression equation of the standard curve (Harmita, 2004). In the test results obtained LOD values 0.124 µg/mL and LOQ 0.412 µg/mL.

c. Precision

Precision is measured by calculating the relative raw deviation (SBR) from multiple repeat measurements. Precision calculations are listed in Table 2.

Table 2. Precision Calculation Results through Variation Coefficient.

Concentration (µg / mL)	Repetition	Average Absorbance	Yi	SD	RSD
6	1	0.2919	0.2925	0.0005	0.1755
	2	0.2929			
	3	0.2926			
8	1	0.3892	0.3890	0.0002	0.0535
	2	0.3889			
	3	0.3888			
10	1	0.4714	0.4715	0.0001	0.0324
	2	0.4715			
	3	0.4717			
12	1	0.6036	0.6033	0.0002	0.0417
	2	0.6034			
	3	0.6031			
14	1	0.7018	0.7018	0.0002	0.0285
	2	0.7016			
	3	0.7020			
Average					**0.0663**

In this research, the value of SBR is 0.0663% and the criteria are very accurate. This shows that this test method can be received with a very accurate precision.

d. Accuracy

Accuracy is the proximity of the measured value to the true value. Testing accuracy is indicated by the percent value of recovery or percent recovery. Penetuan accuracy value is done on five different concentrations. The result of accuracy parameter test in this research listed in table 3.

Tabel 3. Accuracy Calculation Results through percent Recovery.

Concentration (µg / mL)	Repetition	Average Absorbance	Concentration	Average Concentration	Percent Recovery
6	1	0.2919	6.1393	6.1426	102.37
	2	0.2929	6.1473		
	3	0.2926	6.1412		
8	1	0.3892	8.0212	8.0103	100.13
	2	0.3889	8.0078		
	3	0.3888	8.0019		
10	1	0.4714	9.6112	9.6084	96.08
	2	0.4715	9.6085		
	3	0.4717	9.6054		
12	1	0.6036	12.168	12.1600	101.33
	2	0.6034	12.165		
	3	0.6031	12.147		
14	1	0.7018	14.068	14.0652	100.46
	2	0.7016	14.068		
	3	0.702	14.060		

The average percent value of recovery is 100.08±2.396%. This method is acceptable because it is within the required range, ie 80-110% (Harmita, 2004).

Quantitative Analysis of Sample

Quantitative analysis of cefixime antibiotic samples was used UV-Vis spectrophotometric method. The prepared sample was measured with a UV-Vis spectrophotometer instrument at its maximum wavelength of 291nm and the result was an absorbance. Absorbance obtained is 0.3656, then incorporated into the standard curve equation y = 0,0517x-0,0249 and obtained cefixime sample level 100, 71%. These results are in accordance with the requirements in the Indonesian pharmacopoeia, and there is no significant decrease in antibiotic levels during the storage process.

CONCLUSIONS

This research can be concluded that cefixime antibiotics used in Tasikmalaya city health center still have good quality. The potential values of antibiotics and cefixime antibiotic levels still fulfill the requirements listed in Pharmacopoeia Indonesia. In the case of this resistance, there was no significant correlation between cefixime antibiotic quality parameters and antibiotic resistance.

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