Antibiotic resistance: Evaluation of levofloxacin treatment in acute respiratory tract infections cases at the Tasikmalaya City Health Center, Indonesia

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ABSTRACT

Acute respiratory tract infections (ARTIs) are an acute inflammation of the upper and lower respiratory tract caused by the infection of microorganisms or bacteria, viruses, without or accompanied by inflammation of the lung parenchyma. The use of antibiotics is one way to treat respiratory diseases. This study aims to determine the level of resistance of levofloxacin antibiotics to clinical isolates from ARTIs patients at the Tasikmalaya Health Center, Indonesia. The stages of the research included rejuvenation of clinical single isolates from ARTIs patients, identification of bacteria, and antibiotic resistance testing using the paper-disc method. The results of resistance tests from 142 single clinical isolates of acute respiratory infections showed that levofloxacin antibiotics had high levels of resistance of 50.0%, 30.95% of resistance with intermediate levels, and 19.04% were still sensitive. Bacterial identification test results showed bacteria that have been resistant to levofloxacin are from the genus Haemophillus, Streptococcus, Corynebacterium, Staphylococcus, and Bordetella. Treatment of ARTIs with the antibiotic levofloxacin shows that there has been a relatively large resistance, where the results of the identification of all bacteria showed the bacteria that cause ARTIs.

Key words: Acute respiratory tract infections, antibiotic resistance, clinical isolates, levofloxacin

INTRODUCTION

Acute respiratory tract infections (ARTIs) are one of the main causes of morbidity and mortality in developing countries.^[1,2] In Indonesia, ARTIs cause mortality of 28% in infants and 15.5% of them are pneumonia. [3] Complaints

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of this disease are one of the main reasons patients consult a doctor, and also more than 60% the reason for the use of antibiotics.^[4] The bacteria that commonly cause ARTIs include Streptococcus haemoliticus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, and Corynebacterium diphtheria. [5] Monitoring in prescribing by doctors and patient compliance in taking antibiotics is an important factor in increasing antibiotic resistance. [6]

Monitoring of antibiotic resistance cases in the city of Tasikmalaya, Indonesia, reported the cases of resistance in the treatment of ARTIs, namely 43.03% resistant to

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ciprofloxacin, cefadroxil 43.03%, and antibiotic amoxicillin 70.25%. Research data were obtained from more than 300 throat swabs from the patients indicated as having ARTIs.^[7,8]

This study aims to evaluate the effectiveness of levofloxacin antibiotics in the treatment of cases of ARTIs. This study uses clinical isolates from the patients obtained from previous studies.

MATERIALS AND METHODS

Clinical isolate samples from previous studies were rejuvenated, and identification was carried out to determine the purity of clinical isolates in accordance with microbiological standards.

Clinical isolate rejuvenation

Clinical isolate samples were inoculated using Ose to the surface of new Mueller Hinton agar (MHA) growth media and incubated at 37°C for 18 h. After that, the identification of bacteria, which includes morphological parameters of the colony: Color, structure of the colony, as well as different hemolytic and morphological characteristics.

Identification of bacteria

Bacterial identification of clinical isolates was carried out by observing bacterial morphology, Gram staining, and biochemical tests. Biochemical tests conducted were a motility test, carbohydrate fermentation test (glucose, maltose, lactose, manose, and saccharose), indole test, triple sugar iron agar (TSIA) test, urease test, methyl red (MR) test, Voges proskauer test, and citrate test.^[9]

Antibiotic resistance test

Antibiotic resistance testing uses the agar diffusion method with paper-disc techniques. Levofloxacin antibiotics are made standard solutions with a concentration of $10\,\mu g/mL$. The bacterium used for this test is *Staphylococcus aureus*. Resistance test results are obtained by comparing the inhibition zone formed with the standard diameter of the resistant inhibition zone. [10]

RESULTS AND DISCUSSION

Bacteria identification results

This test was carried out to determine the bacterial genus contained in clinical isolates, which included its morphological form, Gram staining test, and biochemical tests.

Bacterial morphological test results

Samples from clinical isolates were grouped into five based on their morphological colonies. Morphological test results from five groups of clinical isolates are shown in Table 1.

Table 1: Morphological test results of clinical isolate samples

Bacteria	a Morphology				
group	Shape	Color	Texture	Edge	
1	Spherical	Yellowish white	Arise convex	Serrated	
2	Spherical, smooth	Yellow	Flat	Jagged	
3	Similar coil	White	Arise crater	Irregular	
4	Spherical	Orange	Flattening	Integral	
5	Dots	Yellow	Flat	Irregular	

Gram staining results

Gram staining test is performed to determine the type of Gram-positive or Gram-negative bacteria. Gram-positive bacteria will turn purple (violet crystals) due to the complex of violet-iodine crystalline dyes being maintained, even though they are given a water-coloring solution to compare fucsin or safranin. While Gram-negative bacteria will be colored red (safranin), this is due to the loss of violet crystal dyes after washing with alcohol.^[11] Gram staining test results from the bacterial group are shown in Table 2.

Biochemical test results

Biochemical tests aim to identify the bacteria in a single-colony isolate sample based on its physiological properties. These physiological properties are related to biochemical activities that occur through bacterial cell metabolism. Biochemical tests carried out in bacterial identification include sugar test (glucose, maltose, mannose, saccharose, and lactose), MR, Vogers-Proskauerindole, urea, motility, TSIA, and Simmon Citrate. [11] The results of biochemical testing of the bacterial group are shown in Table 3.

Resistance test results for levofloxacin antibiotics

Resistance test is a test to determine the activity of bacteria to survive the effects of antibiotics. This test uses a paper-disk method with a sample of clinical bacterial isolates from the mouth smears of patients diagnosed with ARTIs.^[12]

The media used for testing this resistance is MHA. This media was chosen because MHA is very good and sensitive to antimicrobial activity. In addition, MHA media provide good and reproducible results. The levofloxacin antibiotic concentration used for this test was $30~\mu g/mL$. [13]

Evaluation of the results of the resistance test is seen from the calculation of the diameter of the inhibitory zone formed due to antibiotic activity. This inhibition zone is a place where bacteria are stunted due to antibacterial activity or antibiotics. [13] Bacterial growth inhibition zone diameter indicates bacterial sensitivity to antibiotics. Inhibition zone measurement results will be compared with the literature to determine the classification of the bacteria tested. The bacterial classification is divided into resistant, intermediate, and sensitive.

Table 2: Gram staining results

Bacteria group	Shape	Gram
1	Coccobacil	Negative
2	Coccus	Positive
3	Coccobacil	Positive
4	Coccus	Positive
5	Coccobacil	Negative

Table 3: Results of biochemical testing on clinical isolates

Biochemical	Group of Bacteria				
test	I	II	Ш	IV	٧
Glucose	+	+	_	_	_
Manose	+	+	+	+	+
Lactose	+	+	_	_	_
Maltose	+	+	+	+	_
Sacrose	+	+	_	+	_
MR	+	+	_	+	_
VP	+	_	_	_	_
Indole	_	_	_	_	_
Motility	_	_	_	_	_
TSIA	+	+	+	+	_
SC	+	+	_	_	_
Ureum	+	+	+	_	_

MR: Methyl red, VP: Vogers-proskauer, TSIA: Triple sugar iron agar, SC: Simmon citrate, +: Means positive results, -: Means negative results

The results of resistance test against clinical isolate bacterial samples showed that as many as 50% of bacterial samples were resistant; 30.95% intermediates, and 19.04% are still sensitive. ¹³ The results of the resistance test are shown in Figure 1.

The graph shows a decrease in the ability of levofloxacin antibiotics to inhibit the growth of bacteria originating from the isolates of ARTIs patients at the health center in the city of Tasikmalaya, Indonesia. This condition can be caused by various factors, including prescription of irrational antibiotics and patient compliance in the use of antibiotics so that it can affect the increase in resistance.^[14]

CONCLUSIONS

The results of the study can be concluded that there has been a case of resistance to the use of antibiotics levofloxacin in the handling cases of ARTIs in the city of Tasikmalaya. The results showed that about 50% of the antibiotic resistance conditions had occurred levofloxacin. Intermediate conditions of 30.95% can develop into resistance. While only 19.04% of levofloxacin antibiotics are still effective in treating ARTIs.

The results of bacterial identification show that bacteria resistant to levofloxacin are from the genus *Haemophillus*, *Streptococcus*, *Corynebacterium*, *Staphylococcus*, and *Bordetella*.

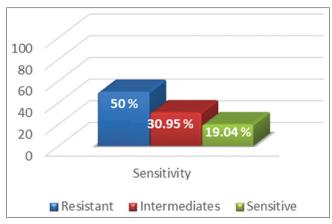


Figure 1: Graph of percentage of resistance clinical isolates of Acute respiratory tract infections patients against levofloxacin antibiotics

The identification of this bacterial genus is in accordance with the literature, which causes ARTIs.[15]

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Conflicts of interest

There are no conflicts of interest.

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