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RESEARCH ARTICLE

Antibiotics Treatment for Acute Respiratory Tract Infections (ARTIs): Evaluation of Doxycycline Resistance Cases at a Community Health Center in Urban Area of Indonesia

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Abstract

Acute respiratory tract infections (ARTIs) are a major health problem in developing countries. This infection can occur in the upper or lower respiratory tract which is accompanied by inflammation. Doxycycline is highly recommended for the treatment of ARTIs patients allergic to penicillin. This study aimed to evaluate the effectiveness of doxycycline in the treatment of ARTIs through its antibiotic resistance rates, and identify bacteria that have been resistant to doxycycline antibiotics. This study consists of the stages of rejuvenation of clinical isolates from ARTIs patients at a community health center in the Urban Tasikmalaya area, identification of bacteria, and testing for antibiotic resistance using the paper-disk method. The results of resistance tests from 116 single clinical isolates showed that there had been resistance to doxycycline antibiotics in the treatment of ARTIs in the urban area of Tasikmalaya with a category of 64.29% being resistant; 14.29% intermediates and 21.42% are still sensitive. The results of bacterial identification showed that doxycycline was resistant to the *Corynebacterium, Streptococcus, Staphylococcus, Haemophillus and Bordetella* bacteria groups. Doxycycline treatment for ARTIs infection is considered not very effective. Another class of drugs as a substitute for doxycycline for patients allergic to penicillin is needed.

Keywords: *Acute respiratory tract infections, Antibiotic resistance, Doxycycline, Clinical isolates.*

Introduction

Acute Respiratory tract infection (ARTIs) is a type of infectious condition that often occurs with a variety of infections and continues to be a major cause of morbidity and mortality in clinical treatment [1]. This infection is usually caused by bacteria such as Streptococcus haemoliticus, Pseudomonas aeruginosa, Bordetella pertussis, Streptococcus pneumoniae, Moraxella catarrhalis, Corynebacterium diphtheriae, and Haemophilus influenzae [2].

In Indonesia, according to basic health research reports the prevalence of RTIs in Indonesia is 25.5% (range: 17.5% - 41.4%) with 16 provinces of which have a prevalence above the National rate.

One of the urban areas with high RTIs potential value is the Tasikmalaya area [3, 4].Doxycycline is the alternative antibiotic of choice in the treatment of ARTIs after the use of first line therapy (amoxicillin) with duration of 10 days of use and failed to respond after 72 hours. Doxycycline is an alternative drug option that is highly recommended especially for patients who are allergic to penicillin [5].

Research conducted by Ramdhani, et al showed that there had been cases of antibiotic resistance in the management of ARTIs in the urban area of Tasikmalaya with resistance levels of Amoxicillin (70.25%), and cefadroxil (69.01%) [6, 7].

This study aims to evaluate the effectiveness of doxycycline antibiotics with clinical isolates of patients obtained in previous studies.

Materials and Methods

The test samples were clinical isolates of ARTIS patients obtained from previous studies.

The clinical isolates were purified to obtain a single isolate, and then rejuvenated. The agar medium used in this resistance test was Mueller Hilton Agar (MHA-OXoid) with a concentration of 38 g/L [8]. Biochemical test materials for bacterial identification include agar (merck), Laltose (merck), mannose (merck), maltose (merck), peptone (Oxoid), phenol red (Taylor), Kovac`s reagent (Bio-Rad), TSIA , methyl red (HiMEdia), α-naphthol (Merck).

Rejuvenation and Purification of Clinical Isolates

Stock clinical isolates obtained from previous studies were rejuvenated by taking bacterial samples with ose and then streaked on new MHA growing media and incubated for 18-24 hours at 37°C. The rejuvenation process aims to remove contamination from bacteria, thereby assisting in the observation process [9]. Purification is done by looking for one isolate without contaminants from other bacteria. Colony morphology observations included color, colony structure, and different hemolytic properties.

Preparation of Test Bacteria Suspension

Preparation of the test bacterial suspension was carried out by inoculating the bacterial colony into a sterile physiological NaCl solution. The turbidity of the bacterial suspension should be equal to the standard turbidity of 0.5 Mc Farland solutions [10].

Antibiotic Resistance Test

The resistance test to clinical isolates used the agar diffusion method, using the paper disc technique. The standard concentration of doxycycline antibiotics used was 30 µg/mL, with the test bacteria *Staphylococcus aureus*. Determination of the resistance value of the tested bacteria to doxycycline was carried out by comparing the diameter of the inhibition zone with the standard diameter of the resistance zone formed [11]. The value of the resistance level category can be seen in Table 1.

Table 1: Categories of doxycycline antibiotic resistance by inhibition zone [11]

Categories	Inhibition Zone (mm)
Resistant	≤ 10
Intermediates	10-13
Sensitive	≥ 14

Identification of Bacteria

Bacterial identification includes determining the morphology of isolates, carried out by observing the color, colony structure, and haemolytic and morphological characteristics of each bacterium. Gram staining was performed to differentiate between Gram positive and negative bacteria using gentian violet carbolic stain [12].

Biochemical test is a follow-up test after determining the morphological characteristics of bacterial colonies. Biochemical tests are used to identify microorganisms physiologically based on their biochemical reactions including: motility test, TSIA (Triple Sugar Iron Agar), urease, Methyl Red (MR) test, Voges Proskauer (VP), Simmon Citrate (SC) and carbohydrate or sugar fermentation. Sugars which include maltose, mannose, saccharose, lactose, and glucose [13]

Results and Discussions

Antibiotic Resistance Test

Antibiotic resistance test of clinical isolates of ARTIs patients used the agar diffusion method, using paper discs containing doxycycline antibiotics. This method was used because it is practical and has good sensitivity in measurement [14]. Mueller Hinton Agar (MHA) is very suitable for antimicrobial susceptibility testing, especially with the paper disc method. In addition, agar media gives good and reproducible results [11].

Observation of the diameter of the inhibition zone which is a clear area shows the presence of antibiotic activity, or shows the sensitivity of bacteria to antibiotics.

The results of the inhibition zone measurement were then compared with the literature to determine the classification of the tested bacteria to be resistant, intermediate and sensitive [11]. Antibiotic resistance test results showed that 64.29% of clinical isolate samples were resistant to doxycycline, 14.29% intermediates, and 21.42% were still sensitive.

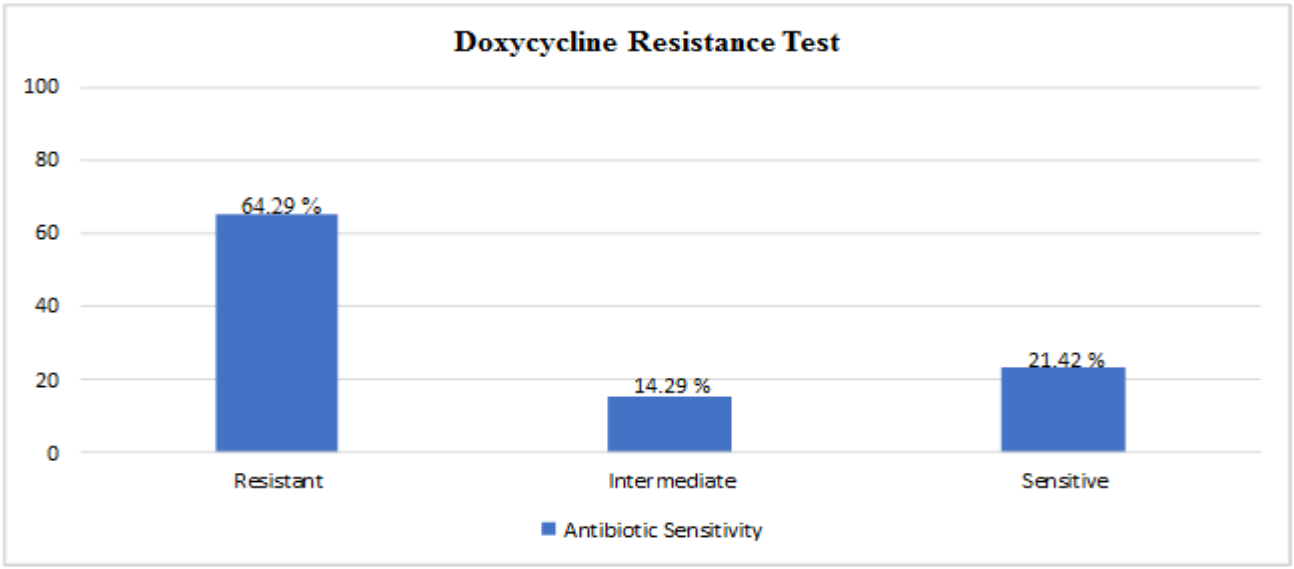

Figure 1: Total percentage of doxycycline resistance test against bacterial isolates

Identification of Resistant Bacteria

Test results resistant bacteria, then identified to determine the genus of bacteria used. Samples were grouped based on their morphology, then another identification test was carried out, the Gram stain test and the biochemical test.

Morphological Test

The morphological test of bacteria was carried out by observing morphology which included size, shape, color and texture [13]. The morphological test results of clinical isolate samples were grouped based on their morphology and obtained five groups of bacteria. The results of morphological observations of the five types of bacteria can be seen in Table 2.

Table 2: Clinical isolate bacterial morphology tests

Group of bacteria	Morphology			
	Shape	Color	Texture	Edge
1	Spherical, thick	Yellowish white	Convex	Jagged
2	Spherical, smooth	Yellow	Flat	Jagged
3	Similar coil	White	Concave	Irregular
4	Spherical	Orange	Embossed	Intact
5	Dots	Yellow	Flat	Irregular

Gram Stain Test

The grouping of bacteria can be seen through differences in the chemical structure of their cell walls, through the Gram stain test. Gram positive bacteria have a thicker cell wall structure, compared to Gram negative bacteria. This is indicated by the purple color results in the Gram stain test due to the ability of the bacterial cell wall to retain the crystal violet-iodine dye [15].The results of gram staining of bacterial groups are shown in Table 3, and Figure 2.

Table 3: Gram strain of bacteria

Group of bacteria	Shape	Gram
1	Coccobacilli	Negative
2	Coccus	Positive
3	Coccobacilli	Positive
4	Coccus	Positive
5	Coccobacilli	Negative

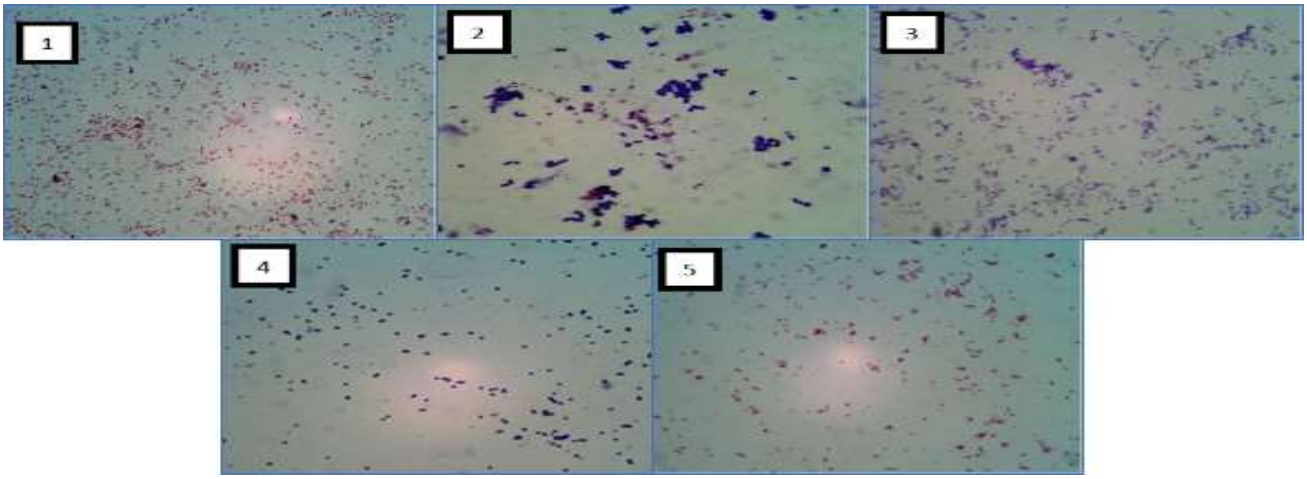

Figure 2: Gram stain of the bacterial group

Biochemical Test

The identification of bacteria through biochemical tests is very useful for distinguishing different bacterial species. This test is determined by the activity of specific enzymes, such as catalase, oxidase, urease, gelatinase, etc., which are produced by bacteria. Biochemical tests can measure the enzyme from a single bacterium in a simple, fast, and generally easy to conclude method. This test can be performed on organisms that have grown in culture and obtained satisfactory results [16]. The results of the motility test, carbohydrate fermentation, indole, TSIA, urease, Methyl Red, Voges Proskauer and citrate test are shown in table 4, and the positive reaction results in Figure 3.

Table 4: Biochemical test of clinical isolates of ARTIs patients

No	Biochemical Test	Group of Bacteria				
		I	II	III	IV	V
1	Motility	-	-	-	-	
2	Glucose	+	+	-	-	-
3	Manosa	+	+	+	+	+
4	Maltose	+	+	+	+	-
5	Lactose	+	+	-	-	-
6	Saccharose	+	+	-	+	-
7	Urea	+	+	+	-	-
8	TSIA	+	+	+	+	-
9	Simmon Citrate	+	+	-	-	-
10	Methyl Red (MR)	+	+	-	+	-
11	Voges Proskauer (VP)	+	-	-	-	-
12	Indole	-	-	-	-	-

(+): Means positive results, (-): Means negative results

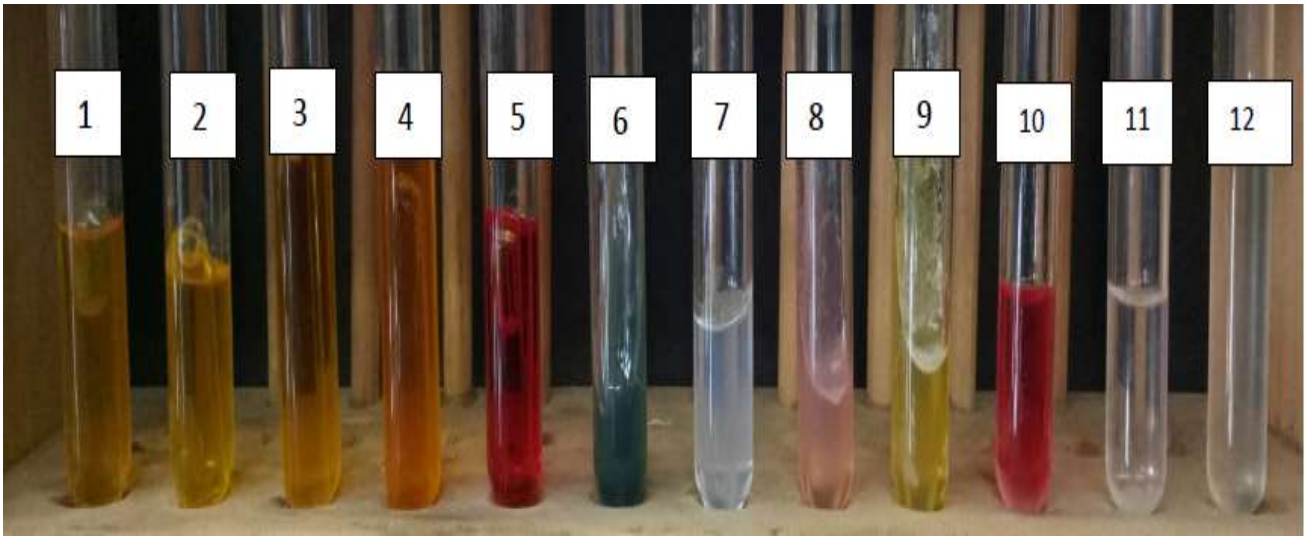

Figure 3: Positive result of biochemical test

Based on the data from a series of identification tests that have been carried out on the five groups of bacteria, it can be seen that phenotypically group 1 is Genus Haemophilus, bacteria group 2 is Genus Staphylococcus, bacterial group 3 is Genus Corynebacterium, bacterial group 4 is Genus Streptococcus and bacterial group 5 is the Genus Bordetella. The five bacterial genera that have been identified are bacterial genera that cause ARTIs disease.

Conclusions

Doxycycline as an alternative antibiotic of choice for ARTIs has decreased its effectiveness. The results of antibiotic resistance tests on stock samples from clinical isolates of ARTIs patients in urban area of Tasikmalaya showed a relatively high resistance value of 64.29%. The identification of the bacterial genus from these isolates showed the conformity of the results with the literature where the genus Haemophilus, Staphylococcus, Corynebacterium, Streptococcus, and Bordetella are the bacterial genera that cause ARTIs.

Acknowledgments

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