Amoxilin resistance in the area of Tasikmalaya, West Java

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ABSTRACT

Acute respiratory infections (ARI) is an acute infection of any part of the respiratory tract and related structures including middle ear, and paranasal sinuses, and pleural cavity. This study was to determine the resistance in isolates of the pathogen of the patients with acute respiratory infections in clinics in Tasikmalaya against antibiotic amoxicillin and effectiveness of this antibiotic. Study was conducted the resistance test against pathogenic isolates of diseased patients ISPA. Results of the patient isolates shows that it is resistance to the antibiotic amoxicillin with the percentage of 70.45%.

Keywords*: Acute respiratory infections (ARI), antibiotic resistance, amoxicillin.*
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INTRODUCTION

Acute respiratory infections(ARI) is a leading cause of morbidity and mortality of infectious diseases in the world. Nearly four million people worldwide die each year due to respiratory infection. The mortality is very high in infants, children, and elderly people, especially in countries with low per capita income and middle [1,2].

ARI is the main target therapies to overcome bacterial infections because bacteria is the etiology of respiratory infection and the second most frequently as a cause of respiratory infection caused by a virus superinfection [3]. In fact, pneumonia accounts for 935,000 under-five deaths annually, representing 15% of all under-five annual worldwide mortality.70% of these deaths occur in just 15 countries in Asia and Sub-Saharan Africa. Many of these countries face significant challenges in the provision of effective health care, diagnosis and treatment. Pneumonia is often misdiagnosed by caregivers in resource-poor settings until it develops into a severe stage [4,5,6]. In Tasikmalaya, Indonesia, the main causes of infant and child mortality are respiratory infections, pneumonia in this case is that prevention and control of ARI is the main priority of health development in Tasikmalaya [7].The number of ARI patients back and forth to the clinic became the basis of this study.

Handling a bacterial infection usually use antibiotics. One of some antibiotics used for the treatment of ARI is amoxicillin. Amoxicillin has a broad spectrum against Gram positive and Gram negative. Amoxicillin works by interfering with the development of microbial cell walls that prevent the action of the enzyme transpeptidase so that bacteria become inactive [8].Currently antibiotic therapy is often misused as a result of irrational use and ease of people to obtain antibiotics without a prescription. It triggers the high multi-drug resistance [9]. The use of antibiotics Repeated and improper is the main cause of the increase in the number of bacteria that are resistant to antibiotics [10.Theoretically, pathogenic bacteria acquire resistance to antibiotics derived from two things, namely by way of vertical transmission and horizontal transmission. In vertical transmission, the bacteria acquire immunity through the accumulation of genetic changes during the natural process of genome duplication while the horizontal transmission transfer of genes from bacteria that mutate into resistant Some of the mechanisms that cause antibiotic resistance is blocking antibiotics by changing the cell wall so it can not be penetrated, changes that lower the target area of connective power of antibiotics, antibiotic-degrading enzymes that produce antibiotics become inactive, lowering the intracellular accumulation of antibiotics by decreasing or increasing the permeability and active efflux of antibiotics [11].

EXPERIMENTAL SECTION

Tools
The tools used in this study is the autoclave (Hirayama), volume micropipette 5-1000 mL (Eppendrof), tip micropipette, incubator (Sakura IF-4), calipers, burners spritus, ose, sterile cotton bud and glassware tools that commonly used in the Laboratory of Microbiology, Faculty of Pharmacy, Universitas Padjadjaran.

Materials
Materials used in this study consisted of antibiotic, bacterial testing and bacterial growth media. Amoxicillin (PT. Kimia Farma), 0.5 McFarland, and physiological saline.

Bacteria Test
Test bacteria used were pathogenic isolates from swabs cavity patients at the health center and health center Taman Sari Cibeureum Tasikmalaya, West Java.

Bacterial Growth Media
Bacteria growth medium used was Mueller Hinton Agar (Merck) with a concentration of 43 g/L and Mueller Hinton Broth (Oxid, Basingstoke, UK) at a concentration of 21 g/L, Mueller Hinton Agar (Merck) with a concentration of 43 g/L.

Method
Sample Size
The study population is drawn from a patient who has been diagnosed by a doctor at the ARI health center. This ISPA patients from patients who seek treatment at Taman Sari Clinic and Cibeureum Clinic, Tasikmalaya, Indonesia, in the period from May to June 2015.

1.   Criteria for inclusion are the patients who were diagnosed with acute respiratory infection by doctors in Taman Sari clinic and Cibereum clinic in the period May to June 2015.
2.   Exclusion criteria that patients with other complications.
The sample size in this study was obtained by using the following formula:

$$N = \frac{(Z\alpha)^2 . P . Q}{d^2}$$

Notes:
N   : sample size
$Z\alpha$: standard deviation
P   : proportion category = 0.5
Q   : 1-P = 0.5
d   : precision = 0.06

In this study, the desired confidence level of 95% so that the value of α was 5% and the Z was 1.96 while the acceptable error (value d) by 6%. Thus the required sample size (N) was 266 samples. In this study sample size used was 332, it aimed to obtain more accurate data so that the research results would be better.

Patient Preparation Oral Swabs
The Swab procedure followed Public Health England, 2014 and modifications other standard methods[12-16]. Patients are first examined by a doctor at the health center, after a positive diagnosis of patients suffering from disorders ISPA then directed to a laboratory to take a swab of the oral cavity upper portion. Making smears performed using sterile cotton buds by analysts moistened with physiological saline solution and then withdrawn at the top of the oral cavity. Cotton buds then applied to the test tube that contains the media to slant to grow bacteria from the oral cavity.

Inoculation of Pathogens
Inoculation is done after the patient's oral swabs were incubated for 18-24 hours at 37°C by taking a swab using a sterile loop and streaked onto agar slant. Incubate back at 37°C for 18-24 hours.

Isolation Technique Pathogens
Isolation of bacteria is done in stages as follows:
A. Making Test Medium
A total of 19 g of Mueller Hinton order was dissolved in 500 mL of distilled water and then sterilized using an autoclave at a temperature of 121° C for 15 minutes. Medium can be stored at 4°C.

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B. Isolation of Bacteria
Pathogenic bacterial isolates from the patient's mouth to be inoculated pathogenic bacteria were isolated by scraping the use ose upward growth media Mueller Hinton Agar. Bacterial isolates of the pathogen would be isolated to form a color, the structure of the colony, as well as the morphology of the same.

Testing Pathogen Resistance Isolates from Oral Swabs[17,18].
Testing of antibiotic resistance through several stages, namely:
A. Sample Preparation
Amoxicillin dosage equivalent to 100 mg were weighed and then added distilled aqua (distilled water) so late, then added a buffer solution of D3 up to 100 mL. Further dilution to reach a concentration of 25 µg/ mL.

B. Resistance Test
Methods for determining the diameter of inhibition zone is the agar diffusion method with paper discs techniques. A total of 20 mL suspension of test bacteria put into a sterile petri dish which contains 20 mL MHA media using micropipette. After that, spread over the surface using a spreader Media MHA. Then put the paper discs containing 20 mL of antibiotics into a petri dish and then incubated at 37°C for 18-24 hours. After that, the inhibition diameter is measured using a caliper formed as a parameter for determining the resistance of bacteria to antibiotic tested.

RESULTS AND DISCUSSION

Sample Test Results
The number of samples obtained swabs from ARI patients from clinics are 332 samples as seen in Table 1.

Table 1.Research Samples

No.	Sample Sources	Fine Samples	Damaged Samples	Description
1	Taman Sari Clinic	63	9	Damaged samples = no growth of bacteria
2	Cibeureum Clinic	245	15	no growth of bacteria
	Total	308	24	

Both the number of samples that can be used to test the resistance and identification totaled 308, while the number of defective samples totaling 24. The sample was a sample which was damaged during the incubation period there was no bacterial growth. This might be caused by several factors such as errors in sampling as clinics officers no cotton buds moistened with sterile distilled water in advance, or cotton buds are not on the surface of the patient's oral cavity and the sample damaged by temperatures that are too hot at the time of transport.

Samples isolates patients with ARI taken from Clinics in Tasikmalaya using test tubes to be skewed. Prior to isolation using test tubes for oblique and resistance tests, all equipment must be sterilized in advance in order to avoid microorganisms that could interfere with the results of testing. To test tube slant that has been sent to the clinics in Tasikmalaya then used a sterile swab tool to take sputum isolates of patients with ARI and streaked onto agar slant that has been provided. Further, the oblique order was brought to the Laboratory of Microbiology, Faculty of Pharmacy, University of Padjadjaran and directly incubated in an incubator. The samples were incubated at 37°C for 18-24 hours and then removed and tested resistance to amoxicillin.

Resistance Test Results
Test of resistance to amoxicillin performed using paper disc method. Paper disc method chosen because this method is very practical versus perforation method. In addition, the paper disc method can also save processing time because the samples used in large numbers. Paper disc method is also more accurate because it uses a fixed concentration in the paper discs which reduce errors while doing resistance tests.Results of the test of resistance can be observed by inhibition zone is formed. What is meant by inhibition zone is a clear area formed around the paper disk which indicated that bacteria can not grow around the paper disc area. This test is intended to determine whether antibiotics are still sensitive to the test bacterium. Inhibition zone accepted as a guide in determining if something is still effective drugs used can be seen in Table 2.

Table 2 Inhibition Zone of Standard Antibiotic [19]

Antibiotic	Concentration in Paper Disc (µg)	Resistant Inhibition Zone	Intermediate Inhibition Zone	Sensitive Inhibition Zone
Amoxicillin	25	<14	15-20	≥21

Samples of bacteria from the oral cavity of patients tested was dissolved in physiological saline so that the color is muddy, bacteria in the solution of NaCl is compared to a 0.5 McFarland turbidity. After that, 20 mL of bacteria in a

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solution of NaCl is taken using a micropipette is inserted into a sterile petri dish that contains 20mL MHA. Bacteria in the solution of NaCl which has been incorporated into the entire portion is flattened so that in a petri dish using a spreader so average. Put each paper disc that contains the antibiotic as much as 20μL using a micropipette.

The paper disc is inserted into an agar medium in a petri dish using tweezers under aseptic state to each of the allotted portion. After incubated for 18-24 hours at 37°C in the incubation chamber. After that, the observed inhibition zone diameter are formed on the surface so that in a petri dish that has been incubated. Inhibitory zone diameters were measured using calipers and read the value of inhibition zone was noted. Antibiotics are used are antibiotic used in Clinics in Tasikmalaya for ARI patients that this study can be seen from antibiotics which are still effective for use in Clinics in Tasikmalaya. Antibiotic used to be reconstituted and diluted by a predetermined dose. The number of samples that are resistant to amoxicillin can be seen in Table 3.

Table 3 Resistance Test Result

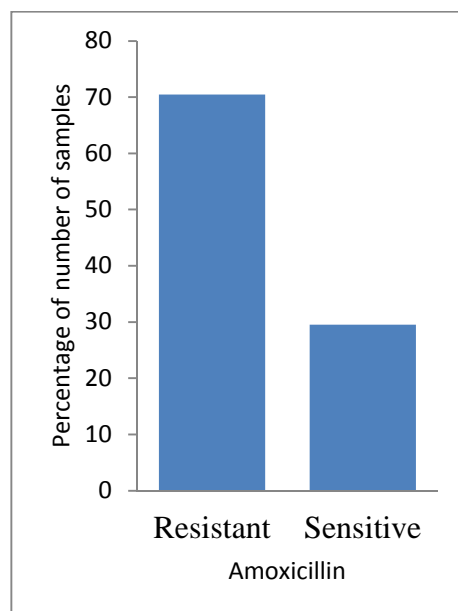| No | Sample | Amoxicillin | |
		Resistant	Sensitive
1	Taman Sari Clinic	40	23
2	Cibeureum Clinic	177	68
Total		217	91

Figure 1. The total number of samples that are resistant or sensitive to amoxicillin in Tasikmalaya

The test results of resistance that can be seen in Table 2 and Figure 1 shows that as many as 308 samples from clinical isolates were tested, the samples that are resistant to amoxicillin 217 samples.

By using Stratified Random Sampling (SRS) technique, the sample was divided into two stratum based on clinics were chosen as the source of the isolates. To determine the proportion percentage of the resistance in Tasikmalaya, researcher used proportion valuation techniques. Estimation for the proportion also involves massive proportions for each stratum. Therefore, through a Stratified Random Sampling of size N, estimation for the proportion is:

$$P_{st} = \sum_{i=1}^{L} \left(\frac{Ni}{N}\right) Pi$$
with $Pi = 1/n \sum_{j=1}^{ni} Xij$
$Xij = 1$ if the sampling unit has the characteristics (resistant)
$Xij = 0$ if the sampling unit has not the characteristic (sensitive)

Based on the data obtained, researcher can determine the proportion is as follows:
$$P_{st} = \left(\frac{63}{308} \; x \; \frac{1}{63} \; x \; 40\right) + \left(\frac{245}{308} \; x \; \frac{1}{245} \; x \; 177\right)$$
$= 0{,}7045$ from 1

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With 95% level of confidence, the percentage of resistance to the antibiotic amoxicillin in Tasikmalaya is 70.45%.It was found that identification of isolates positive samples showed Staphylococcus sp, Staphylococcus aureus, Streptococcus haemoliticus α, β haemoliticus Streptococcus, Streptococcus γ haemoliticus. This type of bacteria that is a real disorder causing bacteria ISPA.

There are several factors that can cause bacterial resistance to antibiotics include changes in the structure of the bacteria so that the bacteria have modified themselves can produce enzymes that can inactivate drugs [20,21]. Another factor that can affect antibiotic resistance is the misuse of antibiotics in which the drug is administered though the indications are not clear. In addition, also due to the improper use of antibiotics and over and repeat [22,23].

CONCLUSION

From this study it can be concluded that there has been resistance in pathogenic isolates from the oral swab of ARI patients in clinics in Tasikmalaya to the antibiotic amoxicillin as much as 70.45%.

It is suggested assessment needs to be carried out in an integrated manner in overcoming resistance amoxicillin antibiotic use in patients with respiratory infections, such as by providing communication, information and education to patients and health workers on the use of antibiotics wisely and enforce rules about the use of antibiotics.

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