
THE SENSITIVITY TEST OF *VIBRIO CHOLERAE* FROM ISOLATION OF DIARRHEA CASE TO VARIOUS TYPE OF ANTIBIOTICS

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ABSTRACT

One of the microbes that causes acute diarrhea is bacteria. *Vibrio cholerae* is one that causes diarrhea called cholera diarrhea. Cholera diarrhea is caused by enterotoxins produced by these bacterial colonies in the small intestine. Giving antibiotics is still the main choice in the treatment and handling of diarrhea because it is expected to kill bacteria and will usually stop diarrhea. Irrational use and the existence of abuse and excessive use of antibiotics can be a factor that causes bacterial resistance to antibiotics. Tests carried out to determine the sensitivity of bacteria to an antibiotic. *Vibrio cholerae* isolate was regrowed in alkaline peptone (APW) water medium and incubated at 37°C for 18-24 hours, then inoculated in the thiosulfate-citrate-bile-sucrose (TCBS) medium. Sensitivity test was carried out on *Vibrio cholerae* bacterial colonies growing on TCBS agar medium by the Disk Diffusion Method from Kirby Bauer. Data were analyzed descriptively. The test results showed that most *Vibrio cholerae* bacterial isolates were still sensitive to all types of antibiotics used in the test. *Vibrio cholerae* has shown resistance to Colistin antibiotics that is equal to 88.2%, Ampicillin at 23.5% and Ceftazidime at 5.9%. The level of resistance to antibiotics that are still low indicates that the antibacterial group can still be used as an alternative in the treatment of cholera diarrhea.

Keywords: *Vibrio Cholerae*; Antibiotics; Resistance.

INTRODUCTION

Diarrhea is characterized by defecation with liquid or semi-solid stools due to more water content than usual. According to the World Health Organization, diarrhea is defecation with a watery or liquid consistency of stool for more than three times a day, while acute diarrhea is diarrhea that occurs suddenly and lasts briefly in a few hours or days.¹ Acute diarrhea due to infection can be caused by bacteria, viruses or parasites. One of the bacterial infections that causes diarrhea is *Vibrio cholerae* and the resulting diarrhea is called cholera diarrhea.² Cholera diarrhea is caused by enterotoxins produced by the bacterial colony in the small intestine. The incubation period for *V. cholerae* infection is commonly 12 to 72 hours. Signs and symptoms that usually come along with cholera diarrhea are vomiting, rice water defecation in large quantities which leads to

dehydration, loss of electrolytes and increased in blood acidity levels. In severe conditions, cholera diarrhea patients will continue to defecate along with vomiting so that it will result in rapid loss of fluid and electrolytes from the digestive tract. If this case is not immediately treated, it may cause death. The death rate due to cholera diarrhea is still quite high, reaching up to 75 percent.^{3,4}

Henceforth, cholera diarrhea is still a serious public health problem. Cholera diarrhea is a plague that has killed millions of people around the world. The high incidence of cholera diarrhea generally occurs in developing countries such as Indonesia. Some risk factors which influence cholera diarrhea are poor hygiene, lack of sanitation and inadequate drinking water supply.⁵ The Directorate for Environmental Control of the Ministry of Health said that at least 30

thousand villages in 440 districts have poor environmental sanitation. *Vibrio cholerae* bacteria enter the human body through foods and drinks that have been contaminated by these bacteria.⁶

Vibrio cholerae is mostly found on the surface of water contaminated with feces that contain these bacteria, thus the environment, especially water plays a major role as a source of infection in cholera outbreaks. Cholera disease prevalence can be an epidemic or extraordinary event (KLB/Kejadian Luar Biasa) that afflicts the people of an area. According to the Regulation of the Minister of Health of the Republic of Indonesia (2004), an extraordinary event (KLB) is an incident of illness or death and / or an increase in an event of illness or death that is epidemiologically significant to a population group within a certain period of time.⁷ Directorate General of Disease Control and Environmental Health The Ministry of Health said in 2008 an outbreak of cholera diarrhea in Timika Papua, in 2009 there was an outbreak of cholera diarrhea in Cisarua Bogor, while in Jember East Java an outbreak of cholera occurred in 2010.^{8,9}

Management that commonly performed in cases of cholera diarrhea are the consumption of solid food and replacement of lost body fluids followed by the use of antibiotics. Giving antibiotics is still the main choice in the treatment and management of diarrhea. Antibiotics that mostly used in the treatment of cholera diarrhea are Tetracycline, Doxycycline or Vibramycin. The administration of antibiotics is expected to kill *V. cholerae* bacteria and will usually stop the diarrhea. A study has reported that the majority (85%) of children who received treatment from Puskesmas in 5 provinces of Indonesia got antibiotics in their prescriptions.¹⁰

Irrational use of antibiotics for therapy are still widely practiced by health workers. In 2011 it was reported that irrational use of

antibiotics was 62.6% to 81.1%. Some literature reported that the *V. cholera* bacteria has been resistant to tetracycline antibiotics. Bacterial resistance to antibiotics can be caused by both misuse and excessive use of antibiotics. Various types of bacteria are now increasingly smart to destroy and outwit the work of antibiotics. In addition, bacteria are also able to destroy the defense mechanisms that should be used by the antibiotics to fight the bacterial infections. This will cause more bacteria with increase their invulnerability. In order to determine resistance level of *V. cholerae* bacteria to certain antibiotics, it is necessary to perform a resistance test. Information about the pattern of *V. cholerae* bacteria resistance to antibiotics has been widely reported, but the pattern of germs and their resistance to antibiotics are varied according to the time and places.^{11,12} Thus, performing a periodical test is important to recognize the sensitivity of pathogenic bacteria to the antibiotics. It is hoped that information about the type of antibiotics that are still effective in the treatment of cholera diarrhea could be obtained through this analysis.

MATERIAL AND METHODS

Sample

The sample that being used on the test of bacterial resistance to antibiotics are isolates of *V. cholerae* obtained from the case of cholera diarrhea. The isolates were stored in the Bacteriology Laboratory of the Center for Biomedical and Basic Technology in Health Research and Development Agency, Ministry of Health of the Republic of Indonesia.

Tools and Materials

The tools and materials used for the inspection include ose needles, test tubes, petri dishes, micro pipettes, blue & yellow tips, erlenmeyer, beaker glass, autoclave, laminar air flow (LAF), spiritus lamps,

incubators, glass objects and other glassware standards, NaCl, peptone, phosphate buffer saline (PBS), 70% alcohol, polyvalent and monovalent of *V. cholerae*, BHI, Mueller Hinton Agar, sterile distilled water, and antibiotic disks. The antibiotic disk used for the resistance test consists of Ciprofloxacin, Ceftriaxon, Norfloxacin, Trimethoprim-sulfametoxazol, Tetracyclin, Ampiciline, Chlorampenicol, Gentamicin, Amicasin, Nalidixid Acid, Colistin, Imipenem, Ceftazidine, and Aztreonamine, and Aztreonamide.

Work Procedure

Vibrio cholerae from isolate stock was regrow in alkaline peptone water (APW) medium and incubated at 37°C for 18-24 hours. The results from the APW medium were then inoculated in thiosulfate-citrate-gall-sucrose (TCBS) medium and incubated at 37°C for 18-24 hours. Colonies that grow on TCBS agar medium are then shaped into specific colonies continued with agglutination tests using polyvalent and monovalent antiserum of *V. cholerae*. The polivalent antiserum of *V. cholera* is used to identify the serogroups, while monovalent antiserum is used to identify the serotypes. Monovalent antisera of *V. cholera* consists of Inaba and Ogawa antiserum. Interpretation of the results of agglutination tests with Ogawa and Inaba antiserum can be seen in Table 1.

Table 1. Interpretation of agglutination test with antiserum of *V. Cholerae*

Serotipe <i>V. cholerae</i> O1	Agglutinasi	
	Antiserum Ogawa	Antiserum Inaba
Ogawa	+	-
Inaba	-	+
Hikojima	+	+

Antibiotic resistance test on *V. cholerae* bacterial colonies that grew on TCBS agar

medium was performed by Disk Diffusion Method from Kirby Bauer (1966). Each bacterial colony that will be tested was put into a liquid BHI medium, then incubated at 37°C for 18 hours. A few drops of suspension were placed into different test tubes along with the physiological NaCl. A sterile cotton swab is inserted into the suspension and pressed to the tube wall. The smeared cotton stick was then spread to the entire surface of the MHA media with a standard thickness of 0.6 cm and allowed to stand for approximately 5 minutes. The antibiotic disc is then carefully placed to the top of the bacterial culture and then gently pressed with sterile tweezers to make sure that it contacts precisely to the tested bacteria, the distance of the disk with the edge of the petri dish is 15 mm and the distance between the antibacterial disks is 24 mm then the culture is incubated for 18-24 hours at 37°C. The formed zones were measured, recorded, and interpreted based on the Performance Standards for Antimicrobial Disk Susceptibility Tests from the National Committee for Clinical Laboratory Standards (NCCLS, 1976). The diameter of the restricted area was compared by the standard antibiotic table. The percentage of *V. cholerae* bacterial resistance was calculated against each type of antibiotic using the equation:

$$\text{Percentage of resistant isolates} = \frac{\text{Numbers of resistant cultures}}{\text{The numbers of cultures tested}} \times 100\%$$

RESULTS

The results of agglutination test with polyvalent antiserum showed agglutination reaction in all *V. cholerae* isolates, this indicates that all of these bacteria belong to the *V. cholerae* serogroup O1. The results of serological tests with monovalent antisera also showed that all of these isolates were Ogawa serotypes of *V. cholerae* O1 (Table 2).

Table 2. The result of agglutination test with antiserum of *V. Cholerae*

Sample Number	Polivalent Antiserum	Polivalen Antiserum	
		Ogawa	Inaba
1	+	+	-
2	+	+	-
3	+	+	-
4	+	+	-
5	+	+	-
6	+	+	-
7	+	+	-
8	+	+	-
9	+	+	-
10	+	+	-
11	+	+	-
12	+	+	-
13	+	+	-
14	+	+	-
15	+	+	-
16	+	+	-
17	+	+	-

Table 3. Percentage of resistance test

Sample Number	Antibiotics	Percentage (%)		
		R	I	S
1	Ciprofloxacin	0	0	100
2	Colistin	88,2	11,8	0
3	Ceftriaxone	0	70,6	29,4
4	Amikasin	0	64,7	35,3
5	Ceftazidime	5,9	0	94,1
6	Norfloxacin	0	0	100
7	Trimethoprim-Sulfamethoxazole	0	0	100
8	Chloramphenicol	0	0	100
9	Imipenem	0	0	100
10	Nalidixic Acid	0	0	100
11	Ampicilin	23,5	64,7	11,8
12	Gentamycin	0	11,8	88,2
13	Tetracyclin	0	0	100
14	Aztreonam	0	88,2	11,8

R : resisten, I : intermediet, S : sensitive

The results of the resistance test showed that most of *V. cholerae* bacterial isolates were still sensitive to all types of antibiotics used in the test. However, the isolated *V. cholerae* has shown a high resistance characteristic that is equal to 88.2% to

Colistin antibiotic, 23.5% to Ampicilin antibiotic, and 5.9% to Ceftazidime antibiotic. The results of *V. cholerae* bacteria resistance test against fourteen types of antibiotics are shown in Table 3 above.

DISCUSSION

The bacteria of *V. cholerae* consists of three serogroups namely *V. cholerae* O1, *V. cholerae* non O1 and *V. cholerae* O139. *Vibrio cholerae* serogroup O1 and O139 are bacteria that cause epidemic and pandemic cholera, whereas *Vibrio cholerae* serogroup non O1 causes cholera-like diarrhea, but the symptoms are still mild and rarely caused an extraintestinal infection. The *V. cholerae* bacteria O1 shows agglutination reaction with O1 antiserum and *V. cholerae* non-O1 which does not show agglutination reaction with O1 antiserum. *V. cholerae* O1 species consists of three serotypes namely Ogawa, Inaba and Hikojima.^{13,14}

In the resistance test, the reading of the results was performed after incubation process for 18 hours at 37°C. By the age of 18 hours, the bacteria are in the active phase, metabolism and enzymes formed optimally and start their phase of pathogenicity. The test of bacterial resistance to antibiotics aims to determine the effectiveness of an antibiotic for the treatment of diarrhea. The use of antibiotics in the treatment of cholera diarrhea is expected to kill *V. Cholerae* bacteria and reduce symptoms of acute dehydration in patients. The case of antibiotic resistance rarely happens when an antibiotic is firstly used. There are two things related to the occurrence of a bacterial resistance to antibiotics, notably the ability of bacteria to form self-defense against antibiotics rapidly and patient factors that help these bacteria to evolve faster. Various types of bacteria are currently getting smarter to disrupt the mechanism of action of the antibiotics. Bacteria are also able to destroy the defense mechanisms that antibiotics should use to

fight the infections. This results in more bacteria with enhanced immunity.^{15,16}

Human factor that become a risk factor for bacterial resistance is inappropriate use of antibiotics. Bacterial resistance to antibiotics may be caused by irrational use of antibiotics. Antibiotics that frequently used in the treatment might lose its effectiveness. Continuous or excessive use of antibiotics can also be a factor that caused bacterial resistance. Irrational use of antibiotics for therapy are still widely practiced by health professionals who prescribe antibiotics for patients that do not necessarily need it, as in the case of viral infections. In addition, incorrect prescription of the antibiotic dosages and the use of broad-spectrum antibiotics to kill bacteria that cause infections will make the targeted bacteria become more resistant to these antibiotics. The use of antibiotics in a long time will give the opportunity for bacteria to become more resistant. Misuse of antibiotic, illustrated by the availability of antibiotics freely on the market without any doctor's prescription and patients who often stop their treatment before its time are also contributed as the factors that affect bacterial resistance. The number of bacteria that become resistant to an antibiotic can increase if the use of antibiotics in the treatment is getting out of control.^{17,18}

The high resistance rate of *V. cholerae* bacteria to antibiotics is attributable to the fact that antibiotics are often used in the treatment for the diarrhea patients, especially children. Another cause of resistance is efflux pumps which play a major role in the expression of virulent genes. Resistance occurs due to changes in the structure of the folate synthetase enzyme with a decrease on its affinity for sulfonamides or loss of permeability. Researchers from the University of New York stated that some pathogenic bacteria could form a kind of nitric oxide which produces enzymes that make these bacteria resistant to antibiotics.

Furthermore, these resistant bacteria quickly multiply and produce new colonies and are increasingly difficult to disable. Resistance can also be caused by mutations in the bacteria itself, particularly a spontaneous mutations in the bacterial chromosomes.^{15,19}

The low level of bacterial resistance to certain antibiotics might be caused by the infrequent use of those antibiotics for handling cholera diarrhea cases. Chloramphenicol could be an alternative choice in the treatment of cholera diarrhea, this is supported by the results of Rahim's study in Bangladesh. Effectiveness occurs as a result of the inhibition process from chloramphenicol to the enzyme peptidyl transferase which acts as a catalyst to form peptide bonds in the process of bacterial protein synthesis.

CONCLUSION

The nature of resistance to chloramphenicol can arise when bacteria are able to form chloramphenicol acetyl transferase enzyme that will damage its activity. The production of this enzyme is controlled by a plasmid. The low level of resistance to antibiotics indicates that those antibacterial group could be potentially used as an alternative choice in the treatment of cholera.^{18,19,20}

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