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Article

Comparison of Sensitivity of Salmonella Typhi Bacteria Isolate Tifoid Fever Patients And Pure Culture To Some Antibiotics In Laboratory

Norma Farizah Fahmi[1], Lelly Aprilia Vidayati[2], Hamimmatus Zainiyah[2] and Nailufar Firdaus[2]

[1]Magister Imunology, Airlangga University, Jl. Airlangga N0. 4-6, Kec. Gubeng, Kota Surabaya, Jawa timur 60115, Indonesia

[2]Midwifery, Ngudia Husada Madura High School of Health Sciences, Jl. RE. Martadinata No. 45, Kec. Bangkalan, Kota Bangkalan, Jawa Timur, 69115, Indonesia

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CORRESPONDENCE

Phone: 085706459343
E-mail: rezaiei.cha@gmail.com

A B S T R A C T

Typhoid fever is one of the infectious diseases which can cause many problems in Indonesia and other developing countries. This fever occurs as a result of infections triggered by Salmonella typhi bacteria. The growth of Salmonella typhi can be inhibited using antibiotics. This study aims at investigating whether there is a difference in the sensitivity test of Salmonella typhi bacteria in an isolate of patients with typhoid fever and pure culture in a laboratory on some antibiotics.

Salmonella typhi bacteria were isolated typhoid fever suspects at one of the hospitals in Surakarta. Pure cultures of Salmonella typhi bacteria were obtained from Microbiological Laboratory of Setia Budi University. Sensitivity test of antibiotics on Salmonella typhi bacteria used diffusion method. Data on antibiotics of inhibition zone diameter (mm) of antibiotics were analyzed statistically using the Two-Way Anova test.

The research results demonstrate that the sensitivity test on Salmonella typhi bacteria in an isolate of patients with typhoid fever shows resistance (R) towards amoxicillin and sensitivity (S) towards trimethoprim, chloramphenicol, gentamicin, ciprofloxacin. Meanwhile, pure culture shows sensitivity (S) towards trimethoprim, chloramphenicol, gentamicin, amoxicillin, ciprofloxacin. The diameter of the inhibition zone of the patient isolate is smaller than that of pure culture.

I. INTRODUCTION

Typhoid fever is an infectious disease which is a problem in the health sector in Indonesia and other developing countries. Typhoid fever can be eradicated by giving antibiotics, vaccinations, and disinfectants. Typhoid fever is an endemic disease that is always present in the community at all times. The World Health Organization (WHO) estimates that the number

of typhoid fever in the world reaches 16-33 million people with 500-600 thousand deaths every year. The high incidence of infectious diseases in developing countries, especially typhoid fever, is associated with the still low socioeconomic status and the low level of knowledge that most people have. People with low socioeconomic status, low nutritional conditions, low knowledge about health, and poor environmental health conditions and poor health status (Masriadi and Susniati, 2013).

Typhoid fever arises from infection by bacteria belonging to the Salmonella group that enters the patient's body through the digestive tract. Salmonella typhi is a rod-shaped bacteria measuring 0.7-1.5 μm x 2.0-5.0 μm, which is Gram-negative so it has a component of the outer layer (outer layer) which is composed of LPS (lipopolysaccharide) and can function as endotoxin, moves with perimetric flagellum, does not form spores. Salmonella typhi has several antigens, among others; O antigen, H antigen, Vi antigen and Outer Membrane especially porin OMP (Darmawati, 2009; Yuliana, 2015).

Salmonella typhi can be inhibited by using antibiotics. Antibiotics are chemicals produced by organisms such as bacteria and fungi, which can interfere with other microorganisms. This antibiotic can kill bacteria (bactericidal) or inhibit the growth of bacteria (bacteriostatic) or other microorganisms. Antibiotics are divided into two, which are active against several species of bacteria (broad-spectrum) and are more specific to certain species of bacteria (narrow spectrum) (Tjay and Rahardja, 2002).

Bacteria originating from typhoid fever patients may have stronger properties than bacteria from microbiological laboratory isolates. Isolates from typhoid fever patients may experience chromosome mutations and/or acquisition of external genetic material so that they are resistant to certain antibiotics (Cita, 2010).

Bacterial resistance to antibiotics can be caused due to the use of antibiotics in a relatively long period of time and continuously so that the bacteria can form a self-defense mechanism if later it will be attacked by the same antibiotic (Sulistianingsih et al (2014).

The researcher wanted to conduct a study on the comparison of the sensitivity test of Salmonella typhi bacteria isolates of typhoid fever patients and pure cultures in the laboratory.

II. METHODS

The sample used was Salmonella typhi bacteria isolated from the blood of patients suspected of typhoid fever in one hospital in Surakarta in January 2017 and pure culture of Salmonella typhi bacteria originating from the Microbiology Laboratory of Setia Budi University Surakarta.

A very extensive antibiotic sensitivity test method is used, namely agar plate diffusion which is also called the Kirby-Bauer agar diffusion test. The sensitivity test of this method is by exposing pure culture to the microorganism tested against an antibiotic disk containing a certain number of antimicrobial agents.

Data obtained from antibiotic sensitivity test diffusion using the Two Way ANOVA method if the Kolmogorov Smirnov test shows that the data is normally distributed. Mann Whitney testing is carried out if the research data is not normally distributed.

III. RESULT

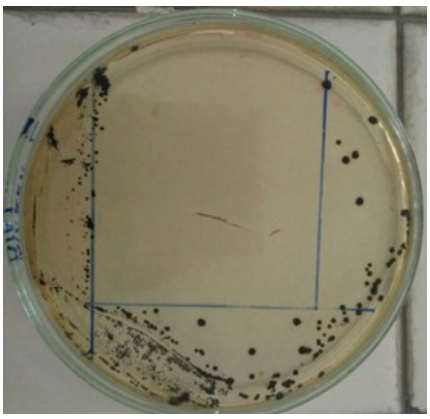


Figure 1. Isolation of Salmonella typhi on SSA media

Isolation of Salmonella typhi from blood samples of typhoid fever patients and pure laboratory cultures. The characteristics of Salmonella typhi colonies on SSA media are round, ± 1-2 mm in diameter, flat/slippery edges, convex elevation, transparent colony color with black core (fisheye). The results of bacterial isolation from the blood of typhoid fever patients and pure laboratory cultures have the same characteristics as Salmonella typhi bacteria, so it can be concluded that these bacteria are suspected of Salmonella typhi bacteria.

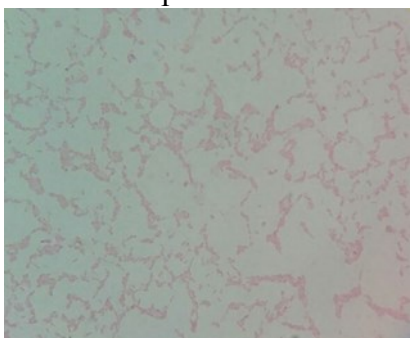
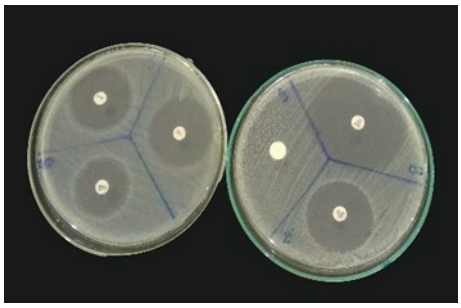


Figure 2. Gram staining of Salmonella typhi bacteria

Gram staining of Salmonella typhi bacteria from the blood of typhoid fever patients and pure laboratory cultures. Rod-shaped bacteria (bacilli), diffuse arrangement, red cell color, transparent pink background, and Gram-negative. The results of Gram staining from colonies that grow on SSA selective media have the same characteristics as Salmonella typhi.

Figure 3. Biochemical Tests Salmonella typhi bacteria

Biochemical Tests Salmonella typhi bacteria from the blood of typhoid fever patients and pure laboratory cultures. MCH media shows bacterial colonies of patient blood isolates and pure K / AS + cultures. The SIM media shows + - + results. LIA media shows the results of K / KS +. Media Simmon's Citrate has positive (+) results. The biochemical test results of the suspected colonies on selective SSA media showed that the bacteria were Salmonella typhi.



Picture 4. Test the sensitivity of Salmonella typhi diffusion methods.

Table 1. The results of the sensitivity test of the diffusion method of Salmonella typhi bacteria on several antibiotics used showed the presence of a clear zone/inhibition zone which was then measured against the diameter of the inhibition zone.

Antibiotik	Repetition	The diameter of antibiotic inhibition zone (mm) and antibiotic sensitivity level			
		Patient isolate	Sensitivity level	Pure culture	Sensitivity level
Trimethoprim	1	21	S	29	S
	2	24	S	30	S
	3	22	S	28	S
Chloramphenicol	1	29	S	32	S
	2	31	S	32	S
	3	31	S	32	S
Gentamicin	1	17	S	25	S
	2	20	S	23	S
	3	21	S	25	S
Amoxicillin	1	20	R	30	S
	2	16	R	30	S
	3	20	R	30	S
Ciprofloxacin	1	35	S	45	S
	2	32	S	39	S
	3	36	S	45	S
Control negative	1	0	-	0	-
	2	0	-	0	-
	3	0	-	0	-

Remarks Table 4: R = Resistance; S = Sensitive

IV. DISCUSSION

Typhoid fever is an acute infectious disease caused by Salmonellah typhi. Typhoid fever is a systemic tropic disease (Rakhman et al, 2009). Salmonella typhi infection into the human body is caused by consuming food or drinks that have been contaminated with this bacteria (Pelczar and Chan, 1988). Examination to diagnose typhoid fever can be done by isolating

bacteria from feces, urine and blood of patients (Irianto, 2014). The diagnosis of typhoid fever can also be done by serology which is a widal method to determine the titer of Salmonella typhi antibodies (Brooks et al, 2001). Typhoid fever is given therapy in the form of antibiotic drugs. This treatment can cause multi drug resistant (MDR) if its use is not rational, so it can change the nature of the Salmonella typhi bacteria found in the human body infected with typhoid fever (Olsen et al., 2004). The difference in average inhibition zone diameter between Salmonella typhi bacteria isolated from typhoid fever patients and pure laboratory culture can be due to differences in the nature of these bacteria. Salmonella typhi bacteria originating from typhoid fever patients have smaller inhibitory zones that can be caused by mutations in the patient's body, as in previous studies that say bacteria that are resistant to antibiotics, there are two types, namely bacteria that are naturally resistant to antibiotics and bacteria that change its nature from sensitive to resistant. Changes in bacterial properties can occur due to chromosome mutations and / or acquisition of genetic material from outside (Cita, 2010).

Bacterial resistance to antibiotics can be caused by the use of antibiotics in a relatively long period of time and continuously so as to enable the bacteria to form a self-defense mechanism if it will be attacked later by the same antibiotic. Treatment with new or rarely used antibiotics, the bacteria need a long time to make a defense mechanism against new antibiotics so that the antibiotics are classified as still sensitive (Sulistianingsih et al, 2014). The mechanism of antibiotic resistance can be categorized namely modification or destruction of antimicrobial agents, pumping antimicrobial agents out of cells by pumping out, modification or replacement of antibiotic targets and decreasing cell membrane permeability. Microorganisms are developing resistance mechanisms by developing mutations in the target protein location gene or obtaining mobile genetic elements that carry resistance genes such as plasmids, integrons and transposons (Abatcha et al., 2014; Ugboko and De, 2014). Amoxicillin is an antibiotic that has a broad spectrum of work, which can be used for Gram negative and Gram positive bacterial infections. The mechanism of action of amoxicillin is by inhibiting cell wall synthesis, which is the initial step of the action of this drug in the form of drug bonding at the cell receptor. B-lactam drugs are attached to one or several receptors, the transpeptidase reaction is inhibited and peptidoglycan synthesis is stopped, then there is a displacement or inactivation of an otolytic enzyme inhibitor in the cell wall. Activation of this lytic enzyme gives rise to lysis if the environment is isotonic. This study shows that Salmonella typhi isolates of typhoid fever patients are resistant to amoxicillin, however pure laboratory culture Salmonella typhi is sensitive to amoxicillin.

Erviani's study (2013) shows the presence of ampicillin or amoxicillin resistance which is a penicillin-derived antibiotic. Amoxicillin resistance is determined by the production of penicillin ring-destroying enzymes produced by the organism (β-lactamase). Beta lactamase opens the β-lactam penicillin ring and β-lactam cephalosporin and eliminates its antimicrobial activity. There is one group of β-lactamases found in gram negative species such as Klebsiella pneumoniae and Escherichia coli. This enzyme is known as Extended Spectrum β-lactamase (ESBL), which has the added ability of bacteria to hydrolyze β-lactam cefotaxime, ceftacidim or astreonam rings. Other Gram negative bacteria have also been reported, namely Enterobacter, Salmonella, Proteus, Citrobacter, Morganella morganii, Shigela dysenterica and Pseudomonas aeruginosa producing ESBL. Salmonella found several types of ESBL, namely enzymes, sulfhydryl variable (SHV), PER, oxacilin OXA and CTX-M. Antibiotics that are

sensitive to Salmonella typhi bacteria isolated from typhoid fever patients and pure laboratory culture are trimethoprim, gentamicin, chloramphenicol and ciprofloxacin. Trimetoprim is an antibiotic that has a broad spectrum of work (Ramadhani, 2012).

This antibiotic has a mechanism of action inhibiting a stage in folic acid metabolism, namely the activity of dehydrofolate reductase, which converts inactive folic acid into a biologically active form of tetrahydro (Tjay and Rahardja, 2002). Previous studies also showed the results of trimethoprim antibiotics are sensitive to Salmonella typhi up to 93.05% (Mulyana, 2007). Gentamicsn also has a broad spectrum of work. Gentamicin is widely used against gram-negative enteric bacteria, especially in bacteremia, sepsis, or endocarditis. Side effects can cause impaired balance and hearing aids (Tjay and Rahardja, 2002). Chloramphenicol has a broad spectrum of action. This antibiotic is widely used for Enterobacter and Staphylococcus aureus by blocking bacterial polypeptide synthesis. Common side effects include gastrointestinal disorders, optic and peripheral neuropathy, inflammation of the tongue and oral mucosa. Previous studies showed that from 2006 - 2010 chloramphenicol was sensitive to Salmonella typhi with a percentage of 100%; 94.3%; 100%; 100%; 96.5% (Alam, 2011). Ciprofloxacin from the group of fluroquinolone can inhibit bacterial cell DNA replication through the blockage of the DNA gyrase enzyme in bacteria, an enzyme that functions to loosen the double chain DNA loops in the DNA replication process. This antibiotic has a narrow spectrum, widely used for gram negative bacteria. Side effects that can occur from the use of antibiotics ciprofloxacin are kidney failure, polyuria, urinary retention, liver disorders, tachycardia, palpitations, hypertension, leukocytopenia, leukocytosis, and others (Tjay and Rahardja, 2002). Previous research shows that from 2006 - 2010 ciprofloxacin was sensitive to Salmonella typhi with a percentage of 100%; 100%; 100%; 100%; 100% (Alam, 2011).

V. CONCLUSION

1. There are differences in the sensitivity test results of Salmonella typhi bacteria isolates of typhoid fever patients, and laboratory pure cultures against several antibiotics, namely:
   a. Trimethoprim antibiotics, gentamicin, chloramphenicol, and ciprofloxacin are sensitive to Salmonella typhi bacteria isolates of typhoid fever patients and pure laboratory cultures.
   b. Amoxicillin antibiotics are sensitive to Salmonella typhi bacteria pure laboratory cultures, but resistant to Salmonella typhi bacteria isolates patients.
2. There are differences in the diameter of the inhibitory zone of Salmonella typhi isolate bacteria in typhoid fever patients and laboratory pure culture of several antibiotics. The diameter of the inhibitory zone of the patient isolates was smaller than pure culture.

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BIOGRAPHY

First Author, I am a woman born in Bangkalan on March 9, 1994. I am the third child from three siblings. I have studied D-IV Health Analyst, Faculty of Health, Setia Budi Surakarta University and graduated in 2016. Since 2016 I have been working at the Ngudia Husada Madura College of Health Sciences for the D-III Laboratory Technology program. Medical. At the moment I am taking the final semester of S2 Immunology education, Unair Postgraduate Faculty. My research interest is about immunology.