PERBANDINGAN SENSITIVITAS *ERYTHROMYCIN* DAN *CHLORAMPHENICOL* TERHADAP BAKTERI *Salmonella* sp.

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ABSTRACT

Salmonella sp. is a digestive pathogenic microbe that potentially causes death. The illness that cause by *Salmonella* sp. infection. is treated with antibiotic therapy. wich thas potentiall temerges problem called antibiotic resistanc. The problem is driven by the misuse of antibiotic therefore appropriate use of antibiotic is needed. This study was carried out to determine the sensitivity of *Salmonella* sp. bacteria towards two kinds of antibiotic with different dosage namely erythromycin 250mg / mL and 500mg/mL and chloramphenicol 250mg/mL and 500mg/mL with 3 replications. The study was conducted by using Kirby-Bauer method (disk diffusion test). *Salmonella* sp. bacteria was suspended in Brain Heart Infusion Broth (BHIB) media and antibiotic tests were carried out with Mueller Hinton agar (MHA) media. The greatest inhibition power was shown by Chloramphenicol 500 mg/mL with 38.6 mm inhibition zone. The following high sensitivity was depicted in the treatment of Chloramphenicol 250 mg/mL with 33.33 mm inhibition zone whereas both of Erythromycin dosages exhibited only intermediate and weak ability in inhibiting *Salmonella* sp. growth.

Key words: Antibiotic Sensitivity Test, Antibiotic Dosage, Inhibition Zone, *Salmonella* sp.

CHAPTER I

INTRODUCTION

1.1. Background of Study

Salmonella sp. is a pathogenic microbe that causes digestive disorders and can result in death, which is known as Salmonellosis (Prasetia, D. I, et al. 2019). The natural habitat of *Salmonella sp.* Is human and animal intestine (Estoepangestie A.T.S., 2014). *Salmonella* bacteria usually move by employing cross-contamination (Melli, F. 2016). *Salmonella sp.* can infect humans once it contaminates food or drink and is then consumed by humans (Reska Perdana, 2016). *Salmonella sp.* is a gram-negative, rod-shaped bacterium that promotes typhoid fever, paratyphoid fever, and indigestion. *Salmonella sp.* that penetrates contaminated food and drink will trigger enteric fever (Dwdjoseputro, 2010; Putu, F. et al, 2018).

Salmonella typhi bacteria or Salmonella paratyphi is the cause of typhoid fever (Sandika, J. 2017). Salmonellosis is transmitted through food and drink contaminated with feces or feces from typhoid fever sufferers (Edi, S. 2018). The mortality rate of this disease is quite high, around 1-5% of sufferers because this disease can be accompanied by various other diseases and causes seriousness (Darmawati, 2009).

Typhoid fever is a health problem in tropical countries, including Indonesia. Globally, the disease caused by typhoid fever reaches 16 million cases per year and 7 million cases in Southeast Asia, with a mortality rate of up to 600,000 per year, and in Indonesia, it ranges from 760-810 cases per 100,000 population with a mortality rate of 3.1-10.4% every year (Nasronudin, 2011).

Typhoid fever in Law number 6 of 1962 on epidemic includes infectious diseases due to contamination from feces infected with *Salmonella sp*. The data from the WHO (World Health Organization) estimate the prevalence rate is around 17 million per year globally, while the mortality rate from typhoid fever reaches 600,000, and 70% of them are found in Asia. According to WHO (World Health Organization) data, the number of typhoid fever patients in Indonesia exceeds 81% per 100,000 population (Indonesian Ministry of Health, 2013).

Antibiotic therapy is an effective treatment for typhoid fever caused by strains of the bacteria *Salmonella sp*. The most commonly consumed antibiotics are Chloramphenicol and Erythromycin from the macrolide group so several strains of *Salmonella sp.* can lead to antibiotic resistance. The emergence of bacterial resistance and even multi-resistance of bacteria to various types of antibiotics prompts various problems in the treatment of diseases due to the bacteria *Salmonella sp*. The pattern of resistance that occurs is highly dependent on the pattern or nature of the bacteria and the use of antibiotics (Juwita, S, et al 2013). Thus, concerns arise over the ineffectiveness of antibiotic therapy. As a result, testing is carried out to investigate the extent of the effectiveness of Erythromycin and Chloramphenicol antibiotics, and the testing for an antibiotic can be conducted scientifically using the method of microbiological testing, namely the sensitivity test. Sensitivity test or antimicrobial susceptibility testing is a technique to determine the sensitivity and resistance of an antibiotic by employing a method of measuring the effect of these compounds on the growth of a microorganism under test.

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Based on the explanation above, the researcher wanted to know how sensitive the bacteria *Salmonella sp.* Is to the antibiotics erythromycin and chloramphenicol. This serves as the foundation for conducting research on antibiotic sensitivity tests on *Salmonella sp.*

1.2. Formulation of Study

Based on the description above, the problems that arise are:

1. Are Erythromycin and Chloramphenicol antibiotics sensitive to *Salmonella sp.* bacteria?

2. Which antibiotic is more sensitive to *Salmonella sp.* bacteria?

1.3. Objective of Study

To find out whether the Erythromycin and Chloramphenicol antibiotics are sensitive to *Salmonella sp*. or otherwise.

1.4.Research Benefits

It is projected that this research can provide benefits:

1. As a source of knowledge to determine the types of antibiotics that are sensitive to *Salmonella sp*.

2. Source of information to the public, doctors, and pharmacists about the use of antibiotics that are sensitive to *Salmonella sp*.

CHAPTER II

LITERATURE REVIEW

2.1 *Salmonella sp.*

Salmonella sp. is a bacterium discovered by Eberth in 1880 and confirmed by Robert Koch in 1881. Although it is a bacterium found in the digestive tract, however, it contaminates the environment, it is generally found in garbage and materials related to fecal contamination (Poeloengan, 2014).

Salmonella sp. is a zoonotic pathogenic bacterium and belongs to the *Enterobacteriaceae*, which is a gram-negative bacillus that causes typhoid fever, paratyphoid, and foodborne diseases. *Salmonella sp.* is a gram-negative rod-shaped bacterium, without spores, without fimbriae, free-moving, has no hoops, has peritrichous flagella, and measures 1-3.5µm x 0.5-0.8µm (Indang, N. 2013). The size of the colonies in the hatching media is on average 2-4mm, with properties of round and transparent colonies and black color in its center, or cloud-like (Radji, M. 2013). *Salmonella sp.* is easy to grow on media and very rarely ferments lactose or sucrose. *Salmonella sp.* grows in aerobic and facultative anaerobic conditions at a temperature of 15°C– 41^0C with an optimum temperature of 37^0C (Ardiansyah et al, 2018).

Salmonella sp. is a microbial pathogen that causes stomach pain and may result in death, which is known as salmonellosis. Salmonellosis is an infection caused by Salmonella that penetrates the body through contaminated food or drink. It can also contaminate water containing *Salmonella sp.* and transmission from one person to another can occur during the infection (National Agency of Drug and Food Control, 2008).

An infected individual will experience acute symptoms of fever, nausea, diarrhea, stomach cramps, dizziness, headache, and vomiting after 12 to 72 hours of infection. These symptoms can last for 7 days. Patients with Salmonellosis can recover without medical treatment, however, some patients can experience very severe diarrhea that requires treatment by doctors. The severity occurs among children with a weak immune system and the duration of the disease depends on the clinical symptoms. *Salmonella sp*. which are pathogenic to humans are *Salmonella typhi, Salmonella parathypi,* and *Salmonella parathypi B* (Radji, 2013)

Salmonella sp. grows aerobically and is also capable of facultative anaerobic growth. *Salmonella sp.* can survive at room temperature and low temperatures for several days and can survive for weeks in waste, dry foodstuffs, pharmaceutics agents, and feces.

According to Ewing, *Salmonella* is classified into 3 species: *Salmonella choleraesuis, Salmonella typhi, and Salmonella enteritidi*s, and bacteria with other genetic types are included in the serotype and *Salmonella paratyphi and enteritidis* are not similar to other new species, such as *Salmonella paratyphi A* is now classified as *Salmonella enteritidis* biosero- *paratyphi* type A (Widagdo, 2012).

The morphology of *Salmonella* bacteria has a non-sporing rod shape, gram-negative characteristics, with a size of 1-3,5 um x 0.8 um, an average colony size of 2-4 mm, and has peritrichous flagella except for *Salmonella pullorum* and *Salmonella galinarium* (Radji, 2013).

2.1.1 Taxonomy of *Salmonella sp.*

Kingdom : *Bacteria*

Division : *Proteobacteria*

Class : *Gammaproteobacteria*

Order : *Enterobacteria*

Family : *Enterobacteriaceae*

Genus : *Salmonella*

Species : *Salmonella typhi, Salmonella paratyphi, Salmonella choleraesuis, Salmonella typhimurium, Salmonella enteridiz, Salmonella Arizona, Salmonella enterica* (Radji, 2010).

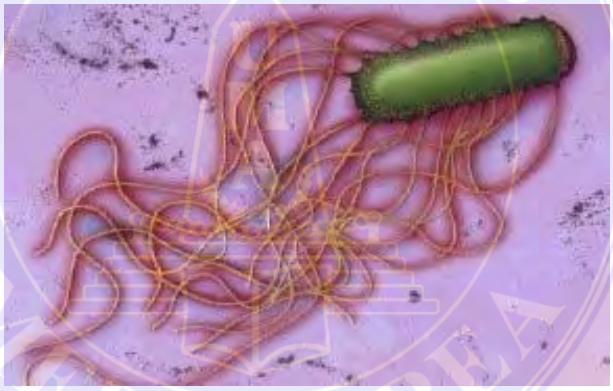

Figure 2.1 *Salmonella sp.* with *Perithric Flagellum* (Source: Rahmawati, 2015)

2.1.2 Physiology of *Salmonella sp.*

Germs grow in aerobic and facultative anaerobic conditions, at a temperature of 15ºC-41ºC (optimum growth temperature is 37.5ºC) and pH of 6-8 (Darmawati, S, 2009). In general, isolates of *Salmonella sp.* are known for their properties, positive motion, negative lactose fermentation reaction, the positive reaction of mannitol, and sorbitol fermentation. On Mac Conkey and Endo Agar

media, they form transparent or clear white colonies because lactose is not fermented (Syahrurachman, A, 2010).

2.1.3 Pathogenicity

The habitat of *Salmonella sp.* is in the digestive system of human beings and animals. Transmission occurs through fecal and oral as a result of consuming contaminated food or drink. Diseases caused by Salmonella are divided into two; nontyphoid salmonellosis (gastroenteritis) and typhoid salmonellosis (typhoid fever).

Several diseases caused by *Salmonella sp.* are as follows:

1. Typhoid Fever

Typhoid fever is an acute infectious disease caused by the bacterium Salmonella typhi, the longest incubation period for typhoid fever among others is generally 10-14 days (Muzadin, L. C et al, 2018). Generally, 10% of patients have recovered from typhoid fever caused by the bacteria Salmonella sp. These bacteria will continue to excrete bacteria through feces for three months and 2-3% can become permanent carriers (Zige et al., 2013; Nadila, P. A. 2017).

These symptoms include fever, constipation, headache, lethargy, rash, loss of appetite, aches, and pains throughout the body, flatulence, nausea, and vomiting. Diarrhea usually occurs during the second week. This condition can be found in the stool of the patient or during the treatment.

2. Gastroenteritis

Gastroenteritis can cause extraordinary events in the community. Approximately 60%-80% of cases appear sporadically due to contaminated food.

Gastroenteritis infection is caused by *Salmonella typhimurium* and *Salmonella enteritidis* or the so-called non-typhoid Salmonella. The incubation period ranges from 12-48 hours or more depending on the symptoms. The initial symptoms are abdominal pain, abdominal cramps, nausea, and vomiting that subside within a few hours, then followed by abdominal pain and fever. Diarrhea is the most prominent symptom. In severe cases, diarrhea can be emitted together with blood (Dwdjoseputro, 2010). Additional symptoms that may appear include muscle weakness and pain, dizziness, nervousness, cold sweats, chills, and restlessness. Patients often recover on their own in 1-5 days but can become acute if there is an electrolyte imbalance and dehydration (Dwdjoseputro, 2010).

2.1.4 Modes of Transmission

The transmission pattern of this disease is through food infected by *Salmonella sp*. that enters through the gastrointestinal tract (mouth, esophagus, stomach, duodenum, small intestine, large intestine). *Salmonella sp.* transmitted through food or drink into the body are *S typhi, S paratyphi* A, B, and C.

When the bacteria *Salmonella sp.* enter the human digestive tract, some of the portions are killed by stomach acid and some *Salmonella sp.* penetrate the small intestine. From the small intestine, *Salmonella sp.* acts so that it can penetrate the small intestine. After successfully damaging the small intestine, *Salmonella sp.* enter the lymph nodes, then into the blood vessels, and throughout the body (especially in the bile, liver, and other organs). In this way, feces containing *Salmonella sp.* is ready to infect other humans through contaminated food or drink. In carrier patients of *Salmonella* bacteria, it can continuously exist in the feces and urine for years (Poeloengan, M. 2014).

From the organs that have been infected, the bacteria will ceaselessly attack through the bloodstream, leading to secondary bacteremia. Secondary bacteremia is responsible for causing fever and clinical disease (Poeloengan, M. 2014).

2.1.5 Epidemiology of *Salmonella sp*.

Contaminated food is the main source of salmonellosis transmission. *Salmonella sp*. can naturally become infected from farm animals such as chickens, turkeys, pigs, cows, or other animals and accommodate bacteria in their tissues. Because *Salmonella sp.* can survive in meat, eggs, and other food products, undercooked food is the main source of transmission of salmonellosis (Poeloengan, M.2014).

Epidemiological studies show that transmission of typhoid fever and other enteric fevers is mainly caused by person-to-person transmission. The transfer of *Salmonella sp*. through water contaminated with feces containing Salmonella sp. is the most common mode of transmission. Identification of *Salmonella sp.* through the determination of DNA fingerprints and phage typing on *Salmonella sp.* is important to carry out when there is an outbreak of salmonellosis to prevent the proliferation of Salmonella in the surrounding environment (Poeloengan, M.2014).

An important factor that needs to be considered in the epidemiology of salmonellosis is the carrier of Salmonella bacteria and a source of transmission of *Salmonella sp*. Therefore, those who have experienced Salmonellosis are not allowed to serve as waiters or prepare food and drinks for others. Another consideration is the use of antibiotics because it can increase the prevalence of

Salmonella resistance to various antibiotics, therefore it will impede efforts to control bacteria once they infect the human body (Poeloengan, M.2014).

2.1.6 Pathogenic factors:

1. Invasion ability

   Salmonella sp. in the small intestine penetrates the epithelium, bacteria move through the epithelial layer into the subepithelial tissue until the lamina propria. The biochemical mechanism at the time of penetration is not clearly understood but a process resembling phagocytosis appears. When bacteria approach the epithelial layer, the brush border degenerates, and then bacteria enter the cells. They are surrounded by an inverted cytoplasmic membrane similar to phagocytic vacuoles. Oftentimes, penetration into the epithelium occurs at the intracellular junction, after which the organism is phagocytized by macrophages, proliferates, and carried by macrophages to other parts of the body (Radji, 2010).

2. Surface Antigen

   The ability of Salmonella to live intracellularly may be due to the presence of surface antigens (antigen vi) (Radji, 2010).

3. Endotoxin

   The role of the endotoxin that may be present in Salmonella infection is not clearly recognized. In experiments using animals, *Salmonella endotoxin* results in various adverse effects, including fever and shock. In experiments using human volunteers who are tolerant to endotoxins infected with *Salmonella typhi*, fever develops as a classic symptom of typhoid fever. This fever is promoted by endotoxin which stimulates the release of pyrogens from macrophage cells and PMN

leukocytes. Furthermore, endotoxin can activate the chemotactic ability of the complement system, which causes the localization of leukocyte cells in small-bowel lesions (Radji, 2010).

4. Enterotoxin

Several species of *Salmonella sp*. produce enterotoxins similar to enterotoxins composed of enterotoxigenic *E. coli* bacteria, both thermolabile and thermostable. *S.typhimurium, S.enteritidis* produce a thermolabile enterotoxin; the toxin is deemed to originate from the cell wall or outer membrane. Toxic activity can be measured by means of rabbit ileal loop and suckling mouse assay (Radji, 2010).

2.2 Antibiotics

Antibiotics are chemical substances produced by microorganisms derived from fungi and bacteria that are effective to inhibit or kill the growth of other microorganisms. Antibiotics are natural compounds produced by living organisms, such as fungi and microorganisms, including derivatives of compounds and their analogous structures that are synthetically created and at low levels are capable of eliminating or inhibiting possible microorganisms (Soekardjo et al., 2008).

Antibiotics are medications used to eradicate or inhibit the growth of microbes, causes of infection among human beings. They are determined to possess the highest possible selective toxicity. Essentially, the antibiotic must be very toxic to bacteria but relatively harmless for humans (Yuliana, A. 2015)

The demand for antibiotics is increasing due to the wide transmission of disease and infection of microorganisms that increasingly propagates (Indrawati,

I. 1017). Antibiotics derive from fungi, microorganisms, and synthetics. Bacterial infection is the biggest health issue in the community, not to mention *Salmonella sp*. that is counterattacked with antibiotics. Antibiotics are drugs that function to kill or slow down the growth of bacteria. Antibiotics, also known as antimicrobials, are substances created by microbes, especially fungi, which are able to eliminate other microbes, such as *Salmonella sp.* These antibiotics can be obtained from fungi and microorganisms unless there are several types made from synthetic and semi-synthetic ones (Pelczar, 2008).

The problem concerned with the use of antibiotic therapy is in the event bacteria are resistant to antibiotics. One of the factors is the inappropriate use of antibiotics (Poelungan, 2010). Antibiotic resistance is the ability of bacteria to survive the effects of antibiotic attacks, which were previously sensitive, however, now are no longer responsive to antibiotics. The effects of antibiotic resistance can lead to prolonged hospital length-of-stay, higher medical expense, and increased mortality (WHO, 2016). . Bacteria become sensitive or resistant to certain antibiotics, consequently, the bacteria will continue to grow and develop, as well as resistant to antibiotics. If bacteria are sensitive to medications, the organisms shall be inhibited and terminated. If bacteria are resistant to a certain antibiotic, the organism continues to grow despite the administration of antibiotics.

2.2.1. Antibiotic Classification

There are three techniques of classifying antibiotics; based on the characteristics, chemical structure, and mechanism of action of bacteria.

1. Based on the characteristics, antibiotics fall into bacteriostatic and bactericidal. Bacteriostatic is the antibiotic property that can inhibit bacterial growth and is temporary (reversible), whereas bactericidal is the antibiotic attribute that can eliminate bacteria and is permanent (Setiabudi, R. 2007). Antibiotics of the bacteriostatic group are chloramphenicol, erythromycin, sulfonamides, tetracycline, trimethoprim, lincomycin, para-aminosalicylic acid, and others, while antibiotics of the bactericidal group are rifampin, isoniazid, penicillins, cephalosporins, cotrimoxazole, aminoglycosides and many more (Isiantoro, 2007).

   The use of bacteriostatic antibiotics works by inhibiting or warding off the growth of bacteria and does not immediately eliminate or eradicate bacteria. There is an increase in the number of a bacterial population so the host's immune system can deal with the bacterial infection. Antibiotics that can actively kill or eliminate bacteria are penicillins, cephalosporins, aminoglycosides (large doses), co-trimoxazole quinolones, rifampin, isoniazid, lactams, and others. However, for those with immune system disorders, bactericidal antibiotics are more favorable since the target of bactericidal antibiotics is capable of killing bacteria (Isiantoro, 2007).

2. Based on the mechanism of antibiotic action that inhibits the metabolism of microbial cells, medications of the cotrimoxazole, para-aminosalicylic acid, sulfonamides, trimethoprim, (PAS), and sulfones. Antibiotics that impede the synthesis of microbial cell walls are affiliated with the group of tetracycline, erythromycin, chloramphenicol, penicillin, cephalosporin, bacitracin, vancomycin, and cycloserine. Antibiotics that may disrupt the

integrity of microbial cell membranes are regarded as part of the group of peptide antibiotics (polymyxin, gramicidin, circulin, tyrosine, valinomycin) and polyene antibiotics (amphotericin, nystatin, Philippines) (Setiwati et al., 2007).

Antibiotics that obstruct the protein synthesis of microbial cells are included in the group of aminoglycosides, macrolides, lincomycin, tetracycline, and chloramphenicol. Common antibiotics that slow down nucleic acid synthesis in microbial cells are quinolones (Ciprofloxacin, Ofloxacin, Moxifloxacin, Levofloxacin, Pefloxacin, Norfloxacin, Sparfloxacin, Lornefloxacin, Flerofloxacin, Gatifloxacin, and rifampin (Setiawati et al., 2007).

3. Given their chemical structure, antibiotics are grouped into the aminoglycoside, including, neomycin, netilmicin, streptomycin, amikacin, gentamicin, kanamycin, and tobramycin. The β-lactams incorporated into the carbapenems are meropenem, ertapenem, and imipenem. The cephalosporins comprise cefazolin, cephalexin, cefuroxime, cefadroxil, ceftazidime, cefotaxime, ceftriaxone, and cefixime, The monocyclic beta-lactams and the penicillins include penicillins, amoxicillin, and ampicillin. Glycopeptides belong to amoplanin, vancomycin, teicoplanin, and decaplanin. Those included in the polyketide group are the macrolide group (erythromycin, clarithromycin, azithromycin, roxithromycin), ketolide group (telithromycin), and tetracycline group (doxycycline, oxytetracycline, tetracycline). The quinolone group is an antibiotic class that is used to treat various bacterial infections and those that belong to the

quinolone group are nalidixic acid, ciprofloxacin, ofloxacin, levofloxacin, and trovafloxacin (Stringer, 2006; Harvey RA, 2013; Pratama, 2014).

The sulfonamides of the sulfonamide group cover cotrimoxazole and trimethoprim. The chloramphenicol group is bound to the ribosomal subunit and impedes the peptidyl transferase enzyme so that peptide bonds are not established in the process of bacterial protein synthesis (Stringer, 2006; Harvey RA, 2013; Pratama, 2014).

2.2.2. Antibiotic Resistance

Resistance is the ability of microorganisms to defeat certain antimicrobials or antibiotics (Djide, N. 2008).

There are two types of host resistance (Pelczar, 1998), namely specific resistance, which directly fights against certain microorganisms, and non-specific or natural resistance.

Several mechanisms cause a bacterial population to become resistant to antibiotics. These mechanisms include changes in the target site, microbes decreasing their permeability so that drugs are difficult to penetrate cells, drug inactivation by microbes, microbes forming shortcuts to avoid processes inhibited by antimicrobials, and increasing the production of enzymes inhibited by antimicrobials (Ganiswarna, et al., 1995).

Based on the origin, bacterial resistance is categorized into two groups (Djide. N, 2006):

1. Genetic resistance consists of spontaneous mutation and transfer of resistance. In spontaneous mutations, microbial genes alter so that microbes that are sensitive to an antibiotic become resistant. This event is

referred to as a spontaneous mutation due to the effect on the antibiotic. Given these antibiotics, selection will occur through a channel that has been resistant to multiplication so that it ends with the formation of a resistant population, whereas the channel that remains sensitive to antibiotics will fight against the bacteria. Resistance transferred from microbes becomes resistance since it obtains a carrier element of antibiotic resistance factors. The transferred resistance factors exist in two forms; plasmid and episome (Djide. N, 2006).

2. Non-genetic resistance is metabolic inactive bacteria which usually are not affected by antimicrobials. This condition is known as non-genetic resistance. These microbes are known as persisters. Microorganisms may exhibit resistance to a drug through a variety of mechanisms as follows (Dwyana, 2006): producing paralyzing enzymes, among these enzymes, include beta-lactamases (penicillinase) which hydrolyze penicillin and transferase enzymes which immobilize aminoglycosides; changing in the structure of the receptor or target molecule (Djide. N, 2006).

   Changes in the structure of the receptor or so-called target molecule require a component of the ribosome to interact, such as erythromycin and aminoglycoside. Changes in the permeability of tetracycline antibiotics are able to accumulate microorganisms that can be affected by antimicrobials, except they cannot be used for resistant microorganisms. The procedure is similar to aminoglycosides, which can be transferred actively into affected cells but cannot enter resistant cells.

Altering metabolic pathways forms alternative metabolic shortcuts (Djide. N, 2006).

The occurrence of bacterial resistance to sulfonamides and fungi that are resistant to flucytosine may arise. Some resistant bacteria to sulfonamides can construct folic acid such as mammalian cells (Djide. N, 2006).

Changes in antibiotic response to several microorganisms result in resistance to trimethoprim by synthesizing large amounts of the enzyme dihydrofolate reductase, which is the objective of the antibiotic action. Decreased receptor activity against aminoglycoside resistance drugs may be associated with the loss or alteration of specific proteins on the bacterial 30S ribosome (Djide. N, 2006).

The increased destruction of these antibiotics is the main mechanism of resistance to penicillins, chloramphenicol, and aminoglycosides. The change of an antibiotic into the active Flucytosine is an antifungal that reforms in microorganisms to fluorouracil, which later is metabolized into the active form of the antibiotic. Fungi become more resistant to flucytosine by shifting enzyme activity along the activation pathway. The cause of microbial resistance can occur vertically (passed down from generation to generation) or frequently horizontally from a donor cell (Djide. N, 2006).

Factors that facilitate the development of resistance to antibiotics include the frequent use of antimicrobials. Frequent use of antibiotics results in a lack of effectiveness of the drug. Next is the inappropriate use of antimicrobials. Many

studies show that inappropriate use of antibiotics is an important factor that facilitates the development of bacterial resistance. The following is excessive use of new antimicrobials. The use of antibiotics for a very long period of time allows bacteria to become resistant (first step mutant). The antibiotics for livestock show that approximately half of the world's antibiotic production is administered to animal feed supplements. Low levels of antibiotics facilitate the growth of bacteria that fight against antibiotics. Several other factors that contribute to the development of resistance are the accessibility to transportation, poor sanitation, sexual behavior, and slum housing conditions (Ganiswarna, 1995).

To anticipate the occurrence of resistance to antibiotics, the use of antibiotics should be remembered. It is wiser to not use antibiotics indiscriminately without ensuring their efficacy and should obtain a prescription from doctors. Antibiotics often used systemically should be avoided as local (topical) drugs. The proper use and duration of antibiotics in any infectious disease that requires antibiotic therapy should be considered. It is recommended that antibiotics be used in appropriate doses depending on the severity of the infection to increase efficacy. Antibiotics used have to be replaced when a disease is resistant (Entjang, I, T 2003).

2.2.3. Use of Antibiotics

The use of antibiotics aims to eradicate the microbes that cause infection. Attention should be addressed to treatment using antibiotics because they possess toxic effects that may harm the body if dispensed in large doses and may cause resistance to uncontrolled long-term use (Oecy, M, et al., 2019). The use of antibiotics is decided based on indications taking into account the factors of the

clinical picture of infectious diseases, which are the effects caused by bacteria in the host. The effect of antimicrobial therapy on infectious diseases is obtained only as a result of the action of antibiotics on bacterial biomechanisms and not on the host's body biomechanisms; antibiotics only slow down the time required by the host body to recover from infectious diseases (Brunton et al, 2006).

Antibiotics that will be transferred to diseases due to bacterial infections can be bacteriostatic or bactericidal, however, the use of antibiotics in humans violating rules will lead to the resistance, not only the bacteria become the target but also other microorganisms in the same habitat as the target microorganism (Waode, 2013).

To determine the administration of antibiotics to an infectious disease, it is necessary to pay attention to clinical symptoms, as well as the ability of the body's immune mechanism. Type and pathogenicity of the bacteria of infectious diseases with mild clinical symptoms do not need to immediately receive antibiotics. Delaying the administration of antibiotics provides an opportunity to stimulate the body's immune mechanism against infection. Yet, a severe symptomatic disease infection, despite it is not dangerous, especially if it has been going on for some time, requires antibiotic therapy (Setiawati et al., 2007).

The antibiotics used to compare antibiotic sensitivity consist of Erythromycin and Chloramphenicol.

1. Erythromycin belongs to the polyketide group

   Erythromycin which belongs to the polyketide group includes the ketolide group (telithromycin), the tetracycline group (doxycycline, oxytetracycline, tetracycline), the macrolide group (erythromycin,

azithromycin, clarithromycin, roxithromycin). Erythromycin is a broad-spectrum macrolide antibiotic that has activity or sensitivity to gram-negative and gram-positive bacteria. Improper use of these antibiotics can lead to resistance to antibiotics (Ganiswara, 2009).

2. Chloramphenicol

   This drug is bound to the ribosomal subunit and inhibits the peptide transferase enzyme so that peptide bonds are not formed in the process of bacterial protein synthesis (Stringer, 2006; Harvey RA, 2013; Pratama, 2014). Chloramphenicol toxicity in the hemopoietic system of mammalian cells is assumed to be related to the mechanism of action of this drug. Chloramphenicol is generally bacteriostatic. At high concentrations, Chloramphenicol is often regarded as bactericidal against certain germs (Ganiswara, 2009).

CHAPTER III

RESEARCH METHOD

3.1 Time and Place

The research was carried out from August to September 2020 at the Health Laboratory Technical Implementation Unit of Medan Regional.

3.2 Research Materials and Tool

Materials

The materials used in this study were strains of *Salmonella sp.* The antibiotics applied in this study were Erythromycin with 250mg/mL and 500mg/mL concentrations, Chloramphenicol antibiotics in 250mg/mL and 500mg/mL concentrations. The media used for growth and dilution were MHA (Mueller Hinton Agar) and BHIB (Brain Heart Infusion Broth).

Tool

The tools used in this study were Erlenmeyer flask, Petri dish, Beaker glass, stirring rod, cotton, dropper, tissue, Bunsen burner, autoclave, aluminum foil, microscope, incubator, spatula, lighter, permanent marker, parchment paper, and sterile cotton, inoculation loop, cloth napkins, latex masks and gloves, labels, and antibiotic sensitivity disk. The instrument for measuring the zone of inhibition of bacteria was a caliper.

3.3 Research sample

The sample used in this research is a strain of *Salmonella* sp.

3.4 Research Method

This research is experimental, the method used is Kirby-Bauer (disk diffusion test) on Mueller Hinton Agar media.

3.5 Work Procedure

Before conducting the research, the tools to be sterilized were initially prepared such as inoculation loop, Petri dish, Beaker glass, stirring rod, and Erlenmeyer flask. Those were washed and put in the oven to be sterile from bacteria and fungi.

3.5.1 Making BHIB (Brain Heart Infusion Broth) Media

Before making the BHIB media, the tools to be used were prepared, then weighed 3 grams of BHIB powder and 100 ml of sterile distilled water were mixed in an Erlenmeyer tube and stirred evenly. Subsequently, the BHIB field was divided into 0.5 ml test tubes and then covered with cotton and sterilized in an autoclave at 121^0C for 15 minutes. After 15 minutes, the media was removed from the autoclave using a cloth napkin.

3.5.2 Preparation of MHA (Mueller Hinton agar) Media

Before making the media, the tools were prepared and MHA media was weighed at 38 grams and 1 liter of aquadest was put into an Erlenmeyer. The media was stirred and heated over a fire/water bath until the media dissolved. After the media dissolved, the Erlenmeyer was covered with cotton which was covered with aluminum foil. Then the Erlenmeyer was put into an autoclave at 121°C for 15 minutes to sterilize the media. After 15 minutes, the media was removed from the autoclave using a cloth napkin, then the sterilized media was cooled to a temperature of 45°C-50°C, and the cold media was poured into sterile Petri dishes and stored at 2^0C-8ºC.

3.5.3 Preparation of Antibiotic test solution

Preparation of antibiotics Erythromycin concentration 250mg/mL, 500mg/mL and Chloramphenicol concentration 250mg/mL, 500mg/mL by placing specimen into test tubes. As many as 10 ml of distilled water was appended and shaken until homogeneous. Subsequently, disks with a diameter of 6 mm were prepared. Following that, samples were soaked in Erythromycin and Chloramphenicol which had been diluted for 15 minutes. Then, they were placed in a petri dish that already contained the media and bacteria (Saudi, 2018).

3.5.4 Preparation of suspension of *Salmonella* sp. and Sensitivity Test

Preparation of bacterial suspension by means of strains of *Salmonella* sp. Was obtained using inoculation loop, put into 0.5 ml of Brain Heart Infusion Broth media, and incubated for 5-8 hours at 37°C. The bacterial suspension was added with sterile distilled water until the turbidity matched the standard 0.5 Mc. Farland I to obtain a germ concentration of 10^8 CFU/ml (CFU: Coloni Forming Unit). Subsequently, a sterile cotton swab was dipped into the bacterial suspension and then pressed against the tube wall so that the cotton was not bathed, then it was smeared on the surface of the Mueller Hinton Agar media evenly (Cavalieri et al., 2005).

To get a homogenous growth, strains of *Salmonella* sp. Were scratched horizontally, then the petri dish was rotated 90^0 and a second stroke was made. Then, the petri dish was rotated 45º and a third stroke was made. After that, the media was dried for 5 minutes. The antibiotic disks of Erythromycin 250mg/mL and 500mg/mL and Chloramphenicol 250mg/mL and 500mg/mL were attached to the MHA and pressed slowly. Following that, it was incubated at 37^0C for 19-24

hours. To measure the diameter of the zone of inhibition of bacterial growth formed around the antibiotic disc, it was measured with a caliper in millimeters. This treatment was repeated 3 (three) times (Kaseng, S, R, et al., 2016).

3.4.5 Data Analysis

In this study, the data were analyzed using One-Way Analysis of Variance (ANOVA) and further tested using DMRT (Duncan Multiple Range Test) because the independent variable and the dependent variable were one, the data used were numerical and there were more than two treatment groups. Requirements to perform ANOVA test The sample came from an independent group. All data analyses were processed using a statistical analysis program, namely the statistical package for the social sciences (SPSS).

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

Based on the results of the study, all things considered, the bacteria *Salmonella* sp. had a strong sensitivity to Chloramphenicol antibiotics at doses of 500mg/mL and 250mg/mL. The sensitivity of the medium category was shown on exposure to Erythromycin at a dose of 500mg/mL, while the antibiotic Erythromycin at a dose of 250mg/mL had weak effectiveness as an antibacterial for *Salmonella* sp., in other words, Salmonella sp. had been resistant to the antibiotic Erythromycin dose of 250 mg/mL.

5.2 Suggestions

It is necessary to conduct research sensitivity tests against *Salmonella* sp. using other antibiotics.