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Research Paper

Analysis of *Escherichia coli Extended-Spectrum Beta-Lactamase* ESBL Bacteria in Chicken Farm Waste in Cipondoh, Tangerang City

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Abstract

Antibiotics are a class of drugs that stop biochemical processes in an organism, stop the growth of bacteria, and naturally create an immune system for the body. The result of using the wrong antibiotics is the occurrence of antibiotic resistance, which means that microorganisms can survive against antibiotics given later. The problem of antibiotic resistance can be spread through livestock, one of which is chicken, one of the most important benefits of chicken farming. In this regard, research was conducted on analysing the number of *E. coli* bacteria in chicken farms in the Cipondoh area, Tangerang City. *E. coli* from livestock waste is one of the microorganisms that can combine with ESBL causing antibiotic resistance. Antibiotic resistance is dangerous if it occurs in humans who consume livestock products and spreads to the environment through the waste discharged into the environment. Antibiotic resistance will cause antibiotics to be unable to prevent disease anymore so that diseases are immune and already resistant to antibiotics. Research on the presence of ESBL bacteria in *E. coli* present in livestock waste needs to be studied further so that the incidence of antibiotic resistance can be prevented and can be followed up as early as possible so that antibiotic resistance or immunity to antibiotics can be prevented as soon as possible and does not become a threat and new epidemics for humankind in the future and humans do not abuse the use of antibiotics against livestock.

Keywords *Antibiotics, Bacterial Resistance, E. coli*

INTRODUCTION

Antibiotics as a class of drugs that are widely used in the fields of medicine and pharmacology. This is because of their benefits in suppressing biochemical processes in an organism. Antibiotics also stop the growth of bacteria (Anggraini et al., 2020). The type of antibiotic most often prescribed by doctors is the penicillin group of antibiotics (ampicillin and amoxicillin). In addition, antibiotics are also used in the livestock industry as drugs to prevent diseases caused by bacteria (Yani et al., 2022). Antibiotics in livestock include beta-lactam groups such as penicillins, cephalosporins, cephamycins, carbapenems, and monobactams.

Antibiotics must be prescribed or under the supervision of a doctor. The wrong of antibiotics will lead to antibiotic resistance. Microorganisms can survive against antibiotics given later because of the presence of resistant genes that arise through mutations and the exchange of plasmids (gene transfer) between the same and/or different bacterial species (Pratiwi, 2017). Bacterial resistance to antibiotics affected to decrease in the effectiveness of therapy and reduced sensitivity (Rukmini et al., 2019). The Centers for Disease Control and Prevention reported that there were million people each year in the United States who detected antibiotics resistant, and approximately 23,000 people died. In 2013 found that about 700,000 deaths worldwide due to antibiotic resistance. It is estimated that in 2050 there will be 10 million deaths due to antimicrobial resistance, with 4.7 million in Asia. Antibiotic resistance could be spread through chickens as common livestock. This is because many chicken farms use antibiotics incorrectly and

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cause chickens to become resistant to antibiotics, then this resistant coding gene spreads through the waste disposed of into the environment and returns to humans through the food chain (Sanders et al., 2013).

Based on Undang-Undang Number 18 the Year 2019 about Animal Husbandry and Animal Health, Livestock is all matters related to physical resources, seeds, seedlings and/or progeny, feed, livestock equipment and machinery, livestock cultivation, harvest, post-harvest, processing, marketing, and business. Livestock is an activity of breeding and cultivating livestock to get benefits and results from these activities (Warsito et al., 2018). One type of livestock in great demand in the market is chicken because chickens can be used all over their bodies for consumption or their eggs. In addition to the many benefits of chicken farming, some problems must be resolved, namely the waste disposal from the chicken farm. Poultry farms produce liquid waste mostly from blood, digestive tract contents, feathers, and residue from washing/cleaning cutting tools. The content of animal slaughter wastewater is organic matter, suspended solids, and colloidal materials such as fat, protein, and high concentrations of cellulose, so livestock wastewater is included in the category of complex liquid waste containing bacteria, one of which is Coliform type Escherichia coli or known as with *E. coli* (Gading et al., 2021). The *E. coli* bacteria contained in chicken farm waste is easily resistant to antibiotics and is dangerous for human welfare because it can produce enzymes resistant to antibiotics. Enzymes that are successfully encoded by antibiotic-resistance genes cause E.coli to become resistant to antibiotics. *E. coli* can spread antibiotic resistance genes to other bacteria, either of the same or different species, horizontally through Mobile Genetic Element (MGE) or vertically through self-division. Livestock waste containing resistant *E. coli* can contaminate other bacteria if it is not treated properly before being disposed of and will return to humans so humans can be infected with these resistant bacteria (Zhang, 2019).

To determine the presence of antibiotic-resistant coding genes, it is necessary to check the amount of *E. coli* contained in livestock waste disposed of into the environment and compare the existing *E. coli* colonies with positive and negative controls for ESBL bacteria or bacteria that are resistant to antibiotics. This is intended as a first step to determine the existence of these resistance genes in *E. coli*. In this regard, research was conducted on analysing the number of *E. coli* bacteria in chicken farms in the Cipondoh area, Tangerang City. From this research, further research can be carried out regarding the specifications of the ESBL bacteria contained in *E. coli* from chicken farm waste in the Cipondoh sub-district, Tangerang City.

LITERATURE REVIEW

Zoonoses are diseases or infections naturally derived between vertebrate animals and humans. Livestock in Indonesia are vulnerable to diseases, including zoonoses. Therefore, zoonoses are new threats to human health (Sukarsih et al., 2008). The development of zoonoses in recent years shows the increasing threat of deadly diseases to humans transmitted by animals. Until now, no less than 300 animal diseases can infect humans. In the past 20 years, 75% of new human diseases have occurred as a result of lockdowns from animal to human or zoonotic and from 1.415 pathogenic microorganisms in humans, 61,6% comes from animals (Sukarsih et al., 2008). Zoonoses can be transmitted from animals to humans in several ways, from direct contact with zoonotic animals and indirect contact through vectors or consuming food from sick livestock or through aerosols in the air when a person is in a polluted environment. Diseases suffered by livestock during rearing can be transmitted to humans through the consumption of food from livestock (Suharsono, 2002)

Broiler farms are generally susceptible to disease attacks caused by viruses, parasitic bacteria, fungi, the environment and one deficiency of nutritional elements (Tamalluddin, 2012). Antibiotics in the livestock industry generally aim to treat livestock, reducing the risk of death and returning livestock to a healthy condition. In the livestock industry, antibiotics are also used as feed additives to stimulate growth (growth promoter), increase production, and increase feed use efficiency (Bahri et al., 2005).

However, using drugs, antibiotics, feed additives or animal growth-promoting hormones that are not as recommended and not in accordance with the dosage set can cause residues in the livestock products produced (Bahri et al., 2005). Consumption of food of animal origin, such as chicken meat containing antibiotic residues, has many negative impacts on health, namely allergic reactions, toxicity, affecting intestinal flora, immune response, and resistance to microorganisms. Besides being harmful to health, antibiotic residues can also affect the environment and economics (Anthony, 1997). The U.S. Agriculture Department inspects meat, poultry, and processed egg products and rarely finds residues at safe levels. Control center and disease prevention in America report that resistant bacteria are increasing and worrying due to administering antibiotics with low doses in the long term. Because of this, the FDA eventually began to limit the use of antibiotics as growth promoters (Friden, 2013).

Extended-Spectrum Beta-Lactamase (ESBL) is a β-lactamase enzyme located within the plasmid and capable of causing bacterial resistance to penicillins, broad-spectrum cephalosporins with oxymino side chains (cefotaxime, ceftriaxone and ceftasidime) and oximino-monobactam aztreonam (but not to cefamycin or carbapenem) via hydrolysis of the amide bond of these antibiotics, but can be inhibited by serine-type β-lactamase inhibitors, namely sulbactam, clavulanate and tazobactam (Bush et al., 2011). Bacterial resistance to β-lactams occurs in three ways: the destruction of the beta-lactamase enzymes in antibiotics, changes in the target of antibiotics, and decreased intracellular uptake of antibiotics. All of these pathways play an important role in antibiotic resistance. However, bacteria that produce β-lactamase and destroy β-lactam are the main cause of resistance. Beta-lactamase produces resistance to antibiotics by breaking down the structure of the antibiotic. Beta-lactamase will open the beta-lactam ring, change the drug's structure, and block the penicillin-binding protein (PBPs) bond (Forbes et al., 2007).

ESBL organisms are reported to be sensitive to cefamycin (cefoxitin and cefotetan) or carbapenems (meropenem or imipenem) (CDC, 2010). The ESBL gene is located on a plasmid, an extrachromosomal piece of DNA essential for bacterial growth. Plasmids are self-replicating and contain virulence factors (resistant genes). The spread of resistance genes via plasmids can occur from bacteria to bacteria in different species (Dierikx, 2013). The characteristics above make ESBL fall into the category of class A β-lactamases (Ghafourian et al., 2014).

There are three main ESBL coding genes namely, TEM, SHV, and CTX-M (Johns et al., 2012). The TEM type of the ESBL gene is a derivative of the β-lactamase types TEM-1 and TEM-2. Unlike TEM-type ESBLs, a minority of SHV-type β-lactamases are SHV-1 derivatives and most go through the conversion of serine to glycine at position 238. Most of the other SHVs undergo a change of lysine to glutamate at position 240. The serine residue at position 238 can hydrolyze ceftazidime, and the lysine residue at position 240 can hydrolyze cefotaxime. Different things were also shown by ESBLs of the CTX-M type which did not have point mutations. CTX-M was identified as derived from Kluyvera spp. and undergoes shape changes in the plasmid (Ghafourian et al., 2014). The CTX-M gene is a new group of ESBLs and has emerged globally in the last decade. CTX-M β-lactamase currently has the highest occurrence rate of any other ESBL type worldwide. The rapid spread of CTX-M type ESBLs produced by *E. coli* is a problem that has received attention from the medical and veterinary fields (Tamang et al., 2014).

The research conducted by Masruroh et al. (2016) showed that the presence of ESBL-producing *E. coli* bacteria isolated from broiler chicken feces in Bogor City was 25%. ESBL-producing *E. coli* contamination can be an important problem for animal and human health. All detected ESBL-producing *E. coli* tend to resist the antibiotic cefotaxime, so these bacteria are assumed to have the CTX gene.

A study by Kawamura et al. (2017) found that ESBL-producing *E. coli* was confirmed to be present in livestock, raw meat, and ready-to-eat sandwiches and vegetables in Algeria and Korea. Meanwhile, contamination of fruit and vegetables with ESBL producers was not found in Switzerland and the UK. Multiple ESBL genetic variants have emerged in both humans and livestock, and different transferable plasmids of different types usually mediate them.

Subsequent research based on research conducted by Yunindika et al. (2022) with the results showing that ESBL-producing *E. coli* was identified in all samples, with the lowest percentage of ESBL-producing *E. coli* at 10.45% and the highest at 39.52% with an average of 17.76%. ESBL-producing *E. coli* has the risk of polluting the environment through effluent from RPH-U/TPA. These bacteria pose a risk of contaminating the environment through effluent from RPH-U/TPA and can be a health threat, especially to people who live along rivers and use river water as a source of daily life.

RESEARCH METHOD

Samples were taken according to SNI 6989.59-2008. The samples taken were liquid (liquid before slaughtering and liquid after slaughtering) and solid samples derived from feces. Then dilution was carried out based on the Global Tricycle Surveillance ESBL *E. coli* (WHO, 2021), each sample was diluted up to 10-5 with sterile PBS 1:9, and duplicates were made. Then isolation and identification of *E. coli* were carried out based on SNI 2897:2008 concerning Methods for Testing Microbial Contamination in Meat, Eggs, Milk, and Their Processed Products. The media used to identify *E. coli* included MacConkay + cefotaxime 1 mg/L, tryptic soy agar slanting, L-EMBA, and biochemical testing was carried out using the IMViC method. Finally, confirm ESBL based on CLSI in 2020 by comparing the results with positive controls (*E. coli* ATCC 25922) and negative controls (K. pneumonia ATCC 700603).

FINDINGS AND DISCUSSION

The use of antibiotics in livestock plays a major role in the development of resistance to commensal and pathogenic bacteria and can increase the risk of humans being infected with bacteria that have developed resistance. The occurrence of resistance resulted in the treatment process due to bacterial infection in humans becoming ineffective and even failing (Maulana et al., 2018). One of the factors causing resistance is the habit of giving antibiotics as a growth factor or Antibiotic Growth Promoter (AGP), which causes livestock to grow faster but can also cause an increase in intestinal organisms that are resistant to antibiotics (Dibner & Richards, 2005). Improper use of antibiotics and dosage also trigger bacterial resistance to various antibiotics resulting in treatment failure in cases of bacterial diseases in livestock (Rosyidi et al., 2018).

ESBL is a β-lactamase enzyme located within the plasmid and capable of causing bacterial resistance to penicillins, broad-spectrum cephalosporins with oxymino side chains (cefotaxime, ceftriaxone and ceftasidime) and oximino-monobactam aztreonam (but not to cefamycin or carbapenem) via hydrolysis of the amide bond of these antibiotics, but can be inhibited by serine-type β-lactamase inhibitors, namely sulbactam, clavulanate and tazobactam (Bush et al., 2011). Bacterial resistance to β-lactams occurs in three ways, namely the destruction of the beta-lactamase enzymes in antibiotics, changes in the target of antibiotics, and decreased intracellular uptake of antibiotics. All of these pathways play an important role in antibiotic resistance. However, bacteria that produce β-lactamase and destroy β-lactam are the main cause of resistance. Beta-lactamase produces resistance to antibiotics by breaking down the structure of the antibiotic. Beta-lactamase will open the beta-lactam ring, change the drug's structure, and block the penicillin-binding protein (PBPs) bond (Forbes et al., 2007).

From the test results of the research conducted, it was found that *E. coli*, which is suspected of producing ESBL, as indicated by the presence of growth on MacConkay agar containing 1 µg/ml cefotaxime with the characteristics of colonies being round, smooth, red in colour, and surrounded by turbid zones. MacConkey agar is a selective medium and can discriminate between lactose-fermenting and non-lactose-fermenting enteric bacteria. Samples suspected of ESBL-producing *E. coli* were also grown on Levine eosin methylene blue agar (L-EMBA) media using the scratch method. Characteristics of colonies suspected to be ESBL-producing *E. coli* on L-EMBA media are shown in metallic green with a black dot in the middle (Leininger et al., 2001). The metallic green colour of L-EMBA indicates a very strong lactose fermentation (Arul et al., 2008). Then proceed, with the biochemical test where this test in the manufacture of bacterial isolates consisting of the Indole, Methyl Red (MR), Voges Proskauer (VP), and Citrate tests which function to ensure that the bacteria contained are really E-coli. Indole Escherichia coli test results yielded positive results, the isolates tested contained tryptophanase enzymes, so that tryptophan was converted to amino into indole, which is indicated by the formation of cherry red rings on the surface of the culture when added with a few drops of Kovac's (Maulana et al., 2018).

CONCLUSIONS

Based on the results of the study, it was concluded that one of the factors causing resistance is the habit of giving antibiotics as a growth factor or Antibiotic Growth Promoter (AGP), which causes livestock to grow faster but can also cause an increase in intestinal organisms that are resistant to antibiotics. Antibiotic resistance due to ESBL is of deep scientific concern. Rapid detection in the clinical laboratory is essential for the judicious recognition of antimicrobial-resistant organisms. Production of Extended-Spectrum β-Lactamase (ESBL) is a significant resistance mechanism that inhibits antimicrobial treatment of infections and poses a serious threat to the currently available antibiotic arsenal. Therefore, ESBL detection as soon as possible is beneficial for living things. In livestock, ESBL detection can be carried out as a deterrent to the use of ESBL bacteria which are usually found in *E. coli* in all farm contents. This also impacts humans so that humans who consume livestock products are protected from ESBL bacteria that cause antibiotic resistance. This also impacts the environment because it can prevent waste from being dumped into the environment contaminated with ESBL.

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