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Detection of Enterobacteriaceae producing Extended Spectrum -Lactamases (ESBL) in Beef Cattle Sold at the Animal Market in Central Lombok

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Abstract: *The Province of West Nusa Tenggara Province (NTB) is known as a source of beef cattle in eastern Indonesia. The use of antibiotics in animal foods can threaten health and cause antibiotic resistance to the normal flora of the animal and humans. One of the causes of antibiotic resistance is extended spectrum β-lactamase (ESBL). The purpose of the study was to detect the prevalence of ESBL-producing Enterobacteriaceae in beef cattle sold in the central Lombok animal market. The faecal samples were collected from beef cattle to 65. Cultured on MacConkey agar supplemented with cefotaxim 1 mg/L, incubated at 37oC for 24 hours. Then the growing colony were tested for ESBL producer by Double Disk Synergy Test (DDST). Identify the type of bacteria by biochemical testing. Identify the type of bacteria by biochemical testing and gram staining. Results: identified showed 1 (1.5%) sample of ESBL positive beef cattle the faecal samples with Escherichia coli bacteria (100%). Through feces, resistant bacteria contained in animal faecal can migrate to the environment and cross-transmission of genetic material between bacteria through water, soil, air, plants, food, humans and food-producing animals.*
Keywords: *ESBL; beef cattle; enterobacteriaceae; animal market*

I. Introduction

The province of West Nusa Tenggara (NTB) is known as a source of beef cattle in eastern Indonesia. In 2017 the total population of cattle in NTB reached 1,128,760 heads based on the NTB Provincial RPJMD (Suryana, 2019). East Praya District is a potential cattle-producing area because it has less productive land which is sufficient as a place for grazing and is used as a source of business. However, the management of the cattle business is still relatively simple. Farmers still sell live cattle to fellow breeders, livestock brokers, agents (pelele), traders, or at most the livestock market in Praya, Central Lombok (Ilham, 2001).

The increasing demand for beef consumption causes beef producers to pay attention to the quality of the marketed beef. In fact, there are still many problems related to the quality and food safety of these animal products. Problems found in the form of contamination by chemicals such as the use of antibiotics (Dewi, 2014). The use of antibiotics in animal food can threaten health and cause antibiotic resistance in the normal flora of the animal body and even humans. The use of antibiotics in livestock food aims to treat livestock, reduce the risk of death, as a feed additive, stimulate growth, and increase production. Feed containing antibiotics will interact with the tissues (organs) in the animal's body, although a small dose of antibiotic will have a chronic effect and remain in the animal's body.

One of the causes of antibiotic resistance is Extended spectrum -lactamase (ESBL). ESBL is a -lactamase enzyme whose ability to cause bacteria to become resistant to penicillins, 1st, 2nd and 3rd generation cephalosporins, and aztreonam (but not to cefamycins and carbapenems) by hydrolyzing antibiotics and inhibited by -lactamase inhibitors such as clavulanic acid (Naelasari, 2018) isolated from food of animal origin, hospital environment, nursing homes, plants, feces, and the community environment (Soedarto, 2016).

Enterobacteriaceae are gastrointestinal flora of humans and animals and has been found in humans, livestock, livestock products and the environment around livestock (Umadevi, 2011). Wittum et al, (2010) described the presence of ESBL in healthy dairy cattle and meat in the United States. 9.5% of milk in dairy farms was positive for ESBL-producing Enterobacteriaceae. Research by Imasari et al (2018) found that 72% of E coli from dairy cow feces and 79.1% from resident feces around farms contained ESBL and Klebsiella pneumoniae and Enterobacter aerogenes respectively 4.2% in the feces of local residents. Effendi (2020) found 5% ESBL-positive E coli isolates were isolated from meat sold in the traditional Surabaya market.

Problems that arise in the form of increasing the spread of resistant bacteria in the livestock environment, between species of cattle, humans, animal markets, traditional markets, cattle slaughterers, slaughterhouses, cow feces in the environment (grass, water, soil, plants) around farms that are contaminated with ESBL. The spread of resistant bacteria in the environment is difficult to handle and control so that its spread is increasingly widespread and difficult to cure. Therefore, it is necessary to identify ESBL-producing bacteria in beef cattle so that their spread can be controlled and free from infectious animal diseases and improve the quality of beef cattle. This study aims to detect the prevalence of ESBL-producing Enterobacteriaceae in beef cattle sold in the Central Lombok animal market.

II. Research Methods

The type of research used is an analytic observational research using a cross sectional study design. The population in this study used all beef cattle sold at the Barabali animal market, Central Lombok. The research sample was feces from the rectal swab of beef cattle at the Praya animal market, Central Lombok. The sample was selected by simple random sampling method. The sample consisted of 65 samples of beef cattle feces that met the inclusion and exclusion criteria. The inclusion criteria were that the cattle were Bali cattle (Bos javanicus domesticus), healthy cattle, and fat bodies, cattle breeders (sellers) willing to participate in the study as evidenced by informed consent. Exclusion criteria are beef cattle breeders (sellers) who are not willing to participate in the study,

The research materials were faecal samples from rectal swabs of beef cattle, antibiotic discs (ESBL confirmation) consisting of Cefotaxime 30 g disk, Ceftazidime 30 g disk, Ceftriaxone 30 g disk, Astreonam 30 g disk, Amoxicillin or clavulanate (30/10 g disk).) and Cefotaxime 1 mg antibiotic powder. Identification of bacteria using Mac conkey agar media, Triple Sugar Iron (TSI) media, glucose phosphate media, citrate agar medium, sulfide indole motility media (SIM) with Kovac's reagent for indole test, Methyl red solution for MR test, 5% alpha naphthol solution and 40% KOH for the VP test. ESBL confirmation using Muller Hinton Agar media.

The research procedure begins with filling in informed consent by the cattle breeder (the seller), then checking the cows based on the type and physical condition (healthy). Next, take the feces by swabbing the rectum on the cow. In the laboratory, samples of cow faeces will be planted on selective media, namely Mac Conkey agar selective media added with 1 mg/L cefotaxime (CTX) agar media (Imasari, 2018). After being incubated for 18-24 hours at 37°C, the colonies that grew were suspected of being ESBL-producing bacteria. Furthermore, a confirmation test of ESBL enzyme production was carried out by means of colonies suspected of producing ESBL confirmed by the Double Disk Synergy Test (DDST) (Naelasari, 2018) carried out by making 0.5 McFarland solution from colonies growing on Mac Conkey-CTX 1

mg/L agar, then implanted evenly on Mueller Hinton Agar using a sterile swab. After a while, a discontaining clavulanic acid, namely AMC (30/10 g) was placed in the center of the plate. Then put a beta lactam disk, namely CAZ disk (30 g), CRO disk (30 g), CTX disk (30 g) and ATM disk (30 g) each at a distance of 15-20 mm from AMC, if there is an increase in the inhibition zone between one of the beta-lactam disks with a clavulanic acid disk is then interpreted as a positive DDST result.

Identification of ESBL-producing Enterobacteriaceae bacteria using biochemical tests on confirmed ESBL-producing colonies, according to the Microbiology textbook, including TSI test, indole and motility test, Methyl Red test, VP test (Voges Proskauer), citrate test (Lindawati, 2015).

III. Discussion

3.1 Results

a. Research Data

This research was conducted from September to October 2021 at the Biomedical Research Unit of the NTB Provincial Hospital. The sample was selected by a simple random sampling method that met the inclusion and exclusion criteria. A total of 65 samples of beef cattle feces were sold at the animal market. The cattle used in this study are Bali cattle (Bos Javanicus) which are sold at the Barabali animal market, Central Lombok.

b. Research Result

Based on the results of the study, in 65 samples of rectal swab of beef cattle, 44 samples did not show the growth of bacterial colonies on selective media, while 21 samples of feces showed bacterial colony growth on Mac Conkey agar selective media with cefotaxime (CTX) 1 mg/L added. agar medium (Figure 1). Confirmation test for Extended Spectrum - Lactamases (ESBL) using the Double Disk Synergy Test (DDST) obtained 1 sample (1.5%) that was confirmed positive for producing ESBL (Figure 2), a positive result was interpreted as an increase in the inhibition zone between one of the beta disks. lactam with a disc containing clavulanic acid. A positive result on DDST indicates the presence of ESBL enzyme production.

Figure 1. *Enterobacteriaceae (Escherichia coli) bacteria grown on Mac Conkey + CTX 1 mg/L Selective Media*

Figure 2. *Confirmation of ESBL-Producing Bacteria by Double Disk Synergy Test (DDST)*

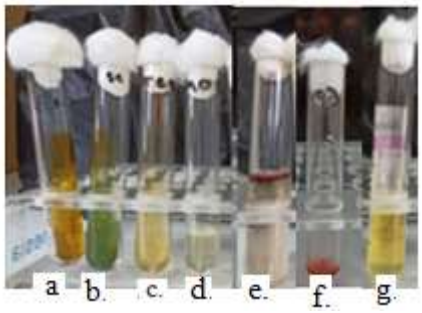

Figure 3. *Biochemical Test of Escherichia coli Bacteria; note: a TSI test; b. Citrate Test; c. Urea Test; d. Motile; e. Indole; f. MR test; g. VP test*

Table 1. Identification of ESBL .-producing Enterobacteriaceae

Types of Bacteria	ESBL		Total %
	Positive n(%)	Negative n(%)	
Escherichia coli	1 (1.5)	64 (8.5)	65 (100)

3.2 Discussion

The results of this study indicate that the incidence of ESBL in beef cattle is still relatively low, namely 1/65 (1.5%) (Table 1). The prevalence of ESBL-producing bacteria is increasing every year, not only in hospitals but these bacteria have spread to the environment and food-producing animals. Beef cattle are one of the food production animals where in this study 1 (1.5%) isolates of beef cattle feces were confirmed to be ESBL positive. Research on ESBL in food-producing animals has also been confirmed, research in Japan conducted by Hiroi et al, 2012 has found ESBL in food-producing animals including Pig 1/33 (3%), beef cattle 2/16 (12.5%), chicken (broiler) 18/30 (60%); dairy cows 3/50 (6%) (Syahputra, 2019), and dairy cows 82/866 (9.5%) (Odenthal, 2016). The emerging resistance is influenced by the irrational use of antibiotics in animals and humans, both for prevention and for the treatment of disease. The use of antibiotics as feed additives for livestock (feed additives) that are used continuously or in small amounts will increase the strain of bacteria that are resistant to antibiotics and can cause disturbances in the normal balance of intestinal microflora (Normaliska, 2019). These ESBL-resistant bacteria are easily transmitted through mobile genetic elements such as plasmids and transposons, besides that there is also resistance to other classes of antibiotics such as fluoroquinolones, sulfamethoxazole, aminoglycosides because one plasmid contains genes for resistance to other classes of antibiotics besides cephalosporins16.

The types of bacteria found in this study were 1 isolate of Escherichia coli (100%) which were identified using biochemical tests. Urea test (-), Motile test positive (+), Indole

test positive (+), Mr test positive (+), VP test negative (-) (Figure 3). The prevalence of Escherichia coli producing ESBL from beef cattle feces in the city of Bogor, Indonesia is 15.8% (Sukmawinata, 2015. Another study conducted by Odenthal et al. in 2016 the results of the incidence of Escherichia coli contamination (75.6%) producing ESBL originating from dairy farms in Germany. Escherichia coli bacteria play a role in the spread of resistance genes through foodborne (Syahputra, 2019). Effendi's research (2020) found 5% of ESBL-positive E coli isolates were isolated from meat sold in the Surabaya traditional market18. Escherichia coli producing ESBL is often associated with the use of antibiotics mixed in animal feed or drinking water, even though the concentration of antibiotics added to animal feed is a low dose, which is in the range of 2.5-12.5 mg/kg (ppm) and has been shown to stimulate resistance to pathogenic bacteria. and commensal bacteria in the digestive tract. Through feces, resistant bacteria contained in animal waste can migrate to the environment and cross-transmission can occur in the surrounding environment where the exchange of genetic material between bacteria can occur through water, soil, air, plants, food, humans and food-producing animals (Naelasari, 2018).

Beef cattle are one of the foods of animal origin that are often consumed by the public, beef cattle sold in the central Lombok animal market have good quality, large and healthy cows, although the results of the study detected 1 isolate of Escherichia coli positive ESBL 1 (1 .5%) the incidence is low. The low prevalence of ESBL in this study is because the cattle rearing system in Lombok carried out by beef cattle farmers is still with the traditional system (grazing) to a more intensive system, namely in cages. the availability of sufficient and fresh forage to meet the daily feed. The results of Putri's research (2021) show that the application of beef cattle maintenance management in the aspect of feeding is quite sufficient as many as 78% of farmers.

IV. Conclusion

Based on the identification of Enterobacteriaceae bacteria, it was found that 1 (1.5%) of beef cattle faeces contained 100% Escherichia coli bacteria producing Extended spectrum beta lactamases (ESBL) which were sold at the animal market in Central Lombok. The incidence of ESBL-producing bacteria in beef cattle is low because beef cattle sold in the Central Lombok animal market have good quality, large and healthy cows with sufficient green feed in the form of natural grass.

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