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ANTIBIOTIC RESISTANCE PROFILE OF *ESCHERICHIA COLI* ISOLATED FROM BEEF SOLD AT SEVERAL WET MARKET IN SIDOARJO, INDONESIA

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ABSTRACT

The purpose of this study was to know antibiotic profile of *Escherichia coli* to several antibiotics. Total of 30 swab samples of beef sold at five different wet markets in Sidoarjo, were purchased from six beef vendors of each wet market. Isolation and Identification of *E. coli* was performed by bacteriological method using BGBB, EMBA, TSIA, and SIM. The antibiotic susceptibility test was done according to Kirby-Baurer method using five antibiotic disks, includes Ceftriaxone 30 µg, Erythromycine 15, Gentamycin 10, Meropenem 10, and Tetracycline 30. The antibiotic resistance profile of *E. coli* showed resistance to Tetracycline 31.5% (6 isolates) and each 5.2% (1 isolates) to Ceftriaxone and Gentamycin, intermediate to 5.2% antibiotics Erythromycine, Gentamycin, and Tetracycline and Susceptible 100% to Meropenem, 94.7% to Ceftriaxon, 89.4% to Gentamycin and 63.1% to Erythromycine antibiotics, and 10.5% to Tertacycline antibiotics.

KEYWORD : *Escherichia coli,* Antibiotic resistance, Beff, Markets, Sidoarjo

INTRODUCTION

Food is the most basic requirement for humans, so food availability needs serious attention both in quantity and quality. Animal products are the main source of nutrition for human growth and life. Every year it is reported that beef as one of the most popular foodstuffs in Indonesia continues to increase, so that the demand for food safety from these products also increases (Andirani *et al*., 2000).

Traditional markets have been synonymous with slums, dirty and careless places. Especially in a part of the market that sells meat, lots of flies and cockroaches fly with dirty and dirty floors. The market is very vulnerable and quite high risk of microbial contamination. Sanitation and cleanliness of the sales environment (market) needs to get the attention of both the traders themselves and the relevant officials to minimize the level of microbial contamination. One of the merchandise traded on the market is meat (Toya and Nengah, 2012).

Meat sold in traditional markets, generally less guaranteed cleanliness because it is done in an open and high temperature so that bacteria can flourish (Setiowati and Mardiastuty, 2009). *Escherichia coli* is a type of bacteria found in food and commonly contaminates meat.

Microbial pollution in food is the result of direct or indirect contamination with sources of microbial pollution such as soil, air, water, dust, digestive tract and respiratory of humans and animals. *Escherichia coli* is a group of coliform bacteria that are used as indicators of pollution and poor sanitary conditions for water and food (Effendi *et al.,* 2019). *Escherichia coli* is a normal flora in the digestive tract of animals and humans so it often spreads through drinking water or food contaminated with human and animal feces and cooking of undercooked meat (Todar, 2005).

In this study using antibiotics Erythromycin, Ceftriaxone, Gentamycin, Tetracycline, and Meropenem. The antibiotic is an antibiotic that is quite dominantly used in Indonesia. As in other countries, the pattern of antibiotic use is widely used inappropriately and has reached excessive levels. The development of antibiotic resistance to

antibiotics is strongly influenced by the intensity of antibiotic exposure in an area, uncontrolled use of antibiotics tends to increase the resistance of germs that were initially sensitive. This is indicated by the increasing pattern of antibiotic sensitivity from year to year (Wibisono *et al.*, 2020).

This study aims to determine whether the *Escherichia coli* bacteria that pollute beef sold in several traditional markets in Sidoarjo show resistance to the dominant antibiotics used in Indonesia. Because, if *Escherichia coli* which is resistant to antibiotics infects humans, it can cause harm to health including treatment failure.

MATERIALS AND METHODS

This research was conducted in February 2018 until March 2018. Sampling in the form of a swab on the surface of beef originating from several traditional markets in Sidoarjo. Subsequent samples were examined at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Airlangga University.

Equipment needed for sampling includes sterile test tubes, handscoons, sterile cotton swabs, ice gel, cool boxes, and test tube covers. Equipment used for isolation, identification, and bacterial sensitivity tests include petri dishes, test tubes, test tube racks, durham tubes, erlenmeyer tubes, loop loops, needle loops, pipettes, incubators, measuring cups, autoclaves, ovens (dry sterilizers) , stirrer, cover glass, object glass, electric scales, bunsen burners, tweezers, label paper, cotton rolls, rulers, water proof markers, aluminum voil, autoclaves, ovens, petri dishes, pipettes, sterile cotton (swabs), incubators, vortex, ice cupboard.

Materials used for sampling include: Peptone Water 1% and ice gel. Materials used for the isolation and identification of Escherichia coli bacteria include: Brilliant Green Bile Broth (BGBB); Merck, Eosin Methylen Blue Agar (EMBA); Oxoid, Triple Sugar Iron Agar (TSIA); Merck, Sulfide Indol Motility (SIM); Oxoid, Buffered Peptone Water (BPW); Merck, aquadest, Kovach reagent, and Alcohol (Effendi *et al.*, 2018).

Materials used to test bacterial sensitivity include Mueller Hinton Agar (MHA); Oxoid, Erythromycin 15 µg antibiotic disk, 10 µg Gentamicin, 10 µg Meropenem, 30 µg Ceftriaxone and 30 µg Tetracyclin. The material used as an antiseptic and disinfectant is 70% alcohol.

This study uses a swab method for sampling. The swab method is a sanitation testing method that can be used on flat, bumpy or hard-to-reach surfaces such as cracks, angles, and gaps (Lukman and Soejoedono, 2009). The swab is composed of a stem or handle (12-15 cm long) with the swab's head made of cotton (0.5 cm to 2 cm diameter).

The sample size in this study was 30 samples taken from 5 markets from several traditional markets in Sidoarjo, namely Sepanjang Market, Waru Market, Larangan Market, Wadungasri Market, and Krian Market. In each market 6 samples were taken from different kiosks. The sampling technique uses the Purposive Sampling method which is a way of taking samples by selecting subjects based on specific criteria set by researchers (Kuntjojo, 2009). Samples on beef are taken from several traditional markets in the Sidoarjo region with criteria: dirty neighborhood or stall beef, dirty meat display, unsanitary merchant hygiene (not using aprons and gloves).

Swab samples on Pepton Water 1% media were then transferred to 9 mL Brillilliant Green Bile Broth (BGBB) media. Culture that grows on BGBB media is inoculated on Eosin Methylene Blue Agar (EMBA) media by streaking on the surface of the agar plate. Then the identification of bacteria is examined microscopically to see the shape and color of the colony. Bacterial identification is done by Gram staining. After that, a typical colony of *Escherichia coli* that grows on Eosin Methylen Blue Agar (EMBA) was confirmed by biochemical properties on Sulfide Indol Motility (SIM) and Triple Sugar Iron Agar (TSIA) media.

Testing the sensitivity of *Escherichia coli* bacteria to antibiotics is done by the disc diffusion method and the interpretation of the results refers to the Clinical and Laboratory Standards Institute (CLSI, 2016).

Escherichia coli bacterial isolate from Eosin Methylen Blue Agar (EMBA) media was taken using loop loop and put in a test tube containing Physiological NaCl, then homogenized by using a vortex until the suspension turbidity was equivalent to MacFarland 0.5 standard. Furthermore, after the equivalent, it is planted on the Mueller Hinton Agar (MHA) plate by inserting sterile cotton swabs into suspension and flattened thoroughly on the surface of the Mueller Hinton Agar (MHA) media plate, after which it is left for 10-15 minutes so the bacteria attach and soak into the media. Discs containing antibiotics are placed on the surface of the media using sterile tweezers and incubated for 24 hours at 37 ^{o}C. After being incubated for 24 hours, the

diameter of the bacterial growth inhibition zone formed around the antibiotic disk was measured using a calipers.

The test results are interpreted using tables that relate to the amount of inhibition zone and the level of resistance to determine the sensitivity of bacteria to several antibotics, grouped into three categories namely Sensitive (S), Intermediate (I) and Resistant (R) based on the recommendations of Clinical and Laboratory Standards Institude (Standard) CLSI).

The research design in this study uses qualitative methods that will produce descriptive data, namely research that aims to make a description of a picture or systematically, factual, and accurate (Nazir, 2009)

RESULTS AND DISCUSSION

Based on the results of the isolation and identification of *Escherichia coli* bacteria from beef swabs taken at five traditional markets in Sidoarjo, 19 of 30 samples or 63% of samples were identified as positive for *Escherichia coli* bacteria. That is because the sale of meat in traditional markets is generally done in an open state and the meat is served in locations that are less guaranteed of cleanliness and high air temperature. Bacteria will grow optimally at a temperature of approximately 37 °C.

Open meat sales can also cause consumers to choose meat by holding it so that the meat can be contaminated and the texture becomes soft which can reduce the quality of the meat. Small cuts of meat sold in the market can increase the amount of microbes on the surface of the meat cuts. This cutting will expand the exposed surface area so that the microbes on the cut surface are easier to get food, water and oxygen and expand the area of penetration so that microbes are easier to breed and more easily damaged meat.

Isolation of *Escherichia coli* bacteria is obtained from beef by means of a swab on the surface of the meat, then planting is carried out on EMBA media which is both selective and differential media. Metallic green is the color of the colony in EMBA because *Escherichia coli* can ferment lactose which results in an increase in acid and media levels (Lindquist, 2004). After fertilizing the EMBA, biochemistry is tested on TSIA. In the TSIA test on the upright and tilted (yellow media), this shows the acidic atmosphere in the upright and tilted part. *Escherichia coli* bacteria can ferment glucose, lactose, and sucrose which makes TSIA test results yellow. Testing of *Escherichia coli* by indole test uses SIM media and then drops with kovach reagent as much as 2-3 drops. Kovach reagent is a solution used for indole testing. The red ring color produced by the kovach reagent on the SIM media is an indicator of the presence of *Escherichia coli*. This change is due to the kovach reagent containing p-dimethylbenzaldehyde which is an indication of bacteria capable of breaking down the amino acid compound tryptopane into the water-insoluble

Table 1. Isolation results and identification of *Escherichia coli* from beef in Sidoarjo market.

No.	Name of Market	Number of sample	Positif *Escherichia coli*	
			Total	Proportion
1	Waru	6	3	50%
2	Wadungasri	6	3	50%
3	Larangan	6	3	50%
4	Krian	6	4	66%
5	Sepanjang	6	5	83%
	Total	30	19	63%

The results of sensitivity test antibiotics for 19 *Escherichia coli* isolates can be seen in Table 2.

Table 2. Resistance test results *Escherichia coli* bacteria samples for antibiotics

No.	Antibiotics	Total of sensitive sample (S)		Total of Intermediate sample (I)		Total of Resistance sample (R)	
		n	%	n	%	n	%
1	*Erythromycin* 15 µg	12	63.1	1	5.2	6	31.5
2	*Ceftriaxone* 30 µg	18	94.7	0	0	1	5.2
3	*Gentamycin* 10 µg	17	89.4	1	5.2	1	5.2
4	*Meropenem* 10 µg	19	100	0	0	0	0
5	*Tetracycline* 30 µg	2	10.5	1	5.2	16	84.2

amino para benzaldehyde compound (Djie *et al.*, 2006).

Table 2 shows the antibiotics found Escherichia coli 100% sensitive to Meropenem antibiotics. 94.7% in Ceftriaxone antibiotics, 89.4% in Gentamycin antibiotics, 63.1% against Erythromycin antibiotics, and 10.5% in Tertacycline antibiotics. *Escherichia coli* isolates which showed 5.2% intermediate interpretation of Erythromycin, Gentamycin, and Tetracycline antibiotics. *Escherichia coli* isolates which had the highest resistance level on Tetracycline antibiotics were 84.2%, as many as 16 samples (84.2%). The results of the resistance test also showed that there was resistance to Erythromycin as many as 6 isolates (31.5%) and 1 isolate on Ceftriaxon and Gentamycin antibiotics. Microbes that are sensitive to an antibiotic can become resistant due to excessive and improper use of antibiotic habits (Rahmahani *et al.*, 2020).

The use of antibiotics that are monotonous and without proper procedures can lead to increased antibiotic resistance to antibiotics and the habit of giving antibiotics to livestock causes cattle to grow faster but also causes an increase in intestinal organisms that are resistant to antibiotics (Widodo *et al.*, 2020). Based on the occurrence of this resistance can be divided into three types, namely the first natural resistance, where microbes from the beginning were not sensitive to antibiotics.

CONCLUSION

Based on the results of the study it can be concluded that proven existence of *Escherichia coli* bacteria by 63% of the total sample of 30 beef sold in five traditional markets in Sidoarjo. The results of resistance tests of 19 *Escherichia coli* isolates found that bacteria were 100% sensitive to Meropenem antibiotics, 94.7% to Ceftriaxone antibiotics, 89.4% to Gentamycin antibiotics, 63.1% to Erythromycin antibiotics, and 10.5% to Tertacycline antibiotics. Escherichia coli isolates which showed 5.2% intermediate interpretation of Erythromycin, Gentamycin, and Tetracycline antibiotics. *Escherichia coli* isolates which had the highest resistance level on Tetracycline antibiotics were 84.2%, 16 samples (84.2%), in Erythromycin antibiotics were 6 isolates (31.5%) and 1 (5.2%) isolates in Ceftriaxon and Gentamycin antibiotics.

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