



DETECTION OF *Enterobacteriaceae* LACTOSE FERMENTER BACTERIA PRODUCING EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) IN FOOD SAMPLES AT SURABAYA

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ABSTRACT

Snacks sold on the side of the road are often purchased because they are practical and cheap. The condition of food sold in the open causes a decrease in food hygiene, so bacteria will easily contaminate, both due to flies infesting it and direct contact with the hands of people around it. One group of bacteria that can cause infection and often contaminate food is *Enterobacteriaceae*. These bacteria can produce Extended Spectrum Beta Lactamase (ESBL) enzymes that cause resistance to various antibiotics (MDR). This research aims to detecting ESBL-producing *Enterobacteriaceae* lactose fermenter bacteria in street food sold on the side of the road in the Dukuh Kupang market area, Surabaya. This study is descriptive observational research with a cross-sectional research design. A total of 50 snack food samples were grown on MacConkey agar containing cefotaxime at four micrograms per milliliter. Then an ESBL confirmation test was carried out using the double disk synergy test (DDST). The Result showed that 13 (26%) samples of snack foods were contaminated. ESBL-producing *Enterobacteriaceae* Lactose Fermenter bacteria are ubiquitous. The most contaminated snack food was cassava, while the non-contaminated snack food was stuffed tofu.

INTRODUCTION

Food is a primary need for every human being because it contains various nutrients that the body needs to produce energy (Nuryani et al, 2017). According to WHO (World

Health Organization), food is all ingredients, both processed and unprocessed, that can be eaten, except water and medicines. Various types of food are sold in various places and we can meet them every day. One type of food that we often encounter is food sold on the side of the road. Usually this food is practical because it is served quickly and the price is relatively cheap (Yustiani et al., 2019). Some examples of street food sold on the side of the road are cilok, fried bananas, risoles, batagor, meatballs and others (Prasetya et al., 2019).

The condition of food sold in the open causes a decrease in the level of food hygiene. Food can easily be contaminated with bacteria, for example because flies are infested, handled by many people or because of saliva droplets from people around (Suryaningsih & Wijayanti 2020). Contaminated food can be a medium that causes disease or is called foodborne disease (Nadifah et al, 2014). WHO estimates that diarrheal diseases due to foodborne disease can kill around 2.2 million people each year. According to the CDC (Centre for Disease Control and Prevention), millions of illnesses occur worldwide every year due to foodborne pathogens (Sofy et al., 2017).

One group of bacteria that can cause diarrhea and often contaminate food is the *Enterobacteriaceae* (Oktaviani & Sulistiyawati, 2022). *Enterobacteriaceae* is a family of bacteria that can be found in the digestive tract of humans and animals as normal flora. Under certain conditions, *Enterobacteriaceae* can be pathogenic or cause diseases such as diarrhea, dysentery, typhoid fever and others (Putri, 2015). Examples of the genus are *Escherichia*, *Klebsiella*, *Enterobacter* which are able to ferment lactose, and *Shigella*, *Salmonella* and *Proteus* which are unable to ferment lactose (Darna et al, 2018).

In terms of medicine, *Enterobacteriaceae* bacterial infections are often difficult to cure. This is because these bacteria are capable of producing extended spectrum beta lactamase (ESBL) enzymes. ESBL enzymes can hydrolyze third and fourth-generation beta-lactam antibiotics, also monobactams (aztreonam) thereby causing multi-resistance to antibiotics (Prasetya, 2017). This study aims to detect *Enterobacteriaceae* lactose fermenter bacteria producing Extended Spectrum Beta Lactamase (ESBL) in street food sold on the side of the road in the Dukuh Kupang market area, Surabaya.

MATERIALS AND METHODS

Sample

Fifty (50) samples of snack foods including pisang goreng, mendoan, pohong or cassava, bakwan, and tahu isi, each food contains ten (10) samples, were studied in the Microbiology Laboratory, Faculty of Medicine, Wijaya Kusuma University, Surabaya.

Identification of ESBL Producing Bacteria

Each food sample was cut into small pieces with sterile scissors as much as 10 grams and then placed in the mortar. The sample was mashed and 20 ml of Trypticase Soy Broth (TSB) medium was added. Then it was homogenized using a vortex and incubated for 4 hours at 37°C. After that, 50 µl was taken and spread onto the surface of McConkey's medium containing 2 µg/ml cefotaxime, then incubated for 24 hours at 37°C. Colonies that grow red or pink in color are suspected to be Enterobacteriaceae bacteria, which are usually ESBL-producing *Escherichia coli*, so it is necessary to carry out the DDST (Double disk Synergy Test) test as a confirmation test for ESBL-producing bacteria.

Double Disk Synergy Test (DDST)

Bacterial suspensions were taken from colonies that grew red or pink in McConkey's medium containing 2 µg/ml cefotaxime, then planted in slanted agar media and incubated for 24 hours at 37°C. After that, the colonies growing on the slanted agar medium were put into a tube containing 5 ml of *Trypticase Soy Broth* (TSB) and the turbidity was calibrated with the McFarland standard of 0.5. Then spread it evenly on Muller Hinton's media and wait for 15 minutes. *Antibiotic Amoxiklav* (AMC) was placed in the center and 20 mm from the midpoint of the disc were placed antibiotics *Ceftazidime* (CAZ), *Ceftriaxone* (CRO), and *Cefotaxime* (CTX). Then incubated for 24 hours at 37°C. Observation of widening of the inhibition zone in the CAZ, CRO, and CTX discs around the AMC-facing disc edge indicated ESBL enzyme production. The widening of the inhibition zone varies and is called the keyhole effect.

RESULTS AND DISCUSSION

A total of fifty food samples were studied, thirteen of which were contaminated with ESBL-producing Enterobacteriaceae Lactose Fermenter bacteria (26%). The type of snack food that is most contaminated with ESBL-producing Enterobacteriaceae Lactose Fermenter bacteria is cassava or pohong. While the type of snack food that is not contaminated is stuffed tofu or tahu isi (**Table 1**).

Table 1. Detection of ESBL producing Enterobacteriaceae lactose fermenter bacteria in snack food samples

Snack Food	ESBL producing <i>Enterobacteriaceae</i> lactose fermenter	
	n	Positive (%)
Pisang goreng	10	2 (20%)
Mendoan	10	2 (20%)
Pohong	10	7 (70%)
Bakwan	10	2 (20%)
Tahu isi	10	0%
Total	50	13 (26%)

Snack foods that are detected to contain *Enterobacteriaceae* Lactose Fermenter bacteria indicate that food hygiene is low, both in terms of the preparation, processing and serving processes. Some things in the preparation process that can increase the risk of bacterial contamination are unclean work clothes, not using protective equipment such as aprons, gloves, head coverings, masks and not washing hands with soap (Jiastuti 2018). In the processing process, the use of raw or unboiled water and cooking utensils that are not washed under running water can also increase the risk of bacterial contamination (Sandika & Mulasari 2019).

In the process of serving, snack food that are served without packaging will cause the food to become easily contaminated with bacteria. Often snacks are not taken using tools, but direct contact with the handler's hands. Then the cover for snacks that is commonly used is oil paper, so that the food is not completely covered which causes food contamination through the gaps in the oil paper. Serving food should use closed containers to prevent contamination from the air or vectors such as flies (Yunus et al, 2015). In addition, the locations for selling street food in the market are of course not only food sellers, but also raw material traders who can be a source of contamination. The market location is narrow and crowded with visitors causing limited selling places and dirty surroundings and increases the risk of bacterial contamination (Islamy et al, 2018).

In this study, the type of snack food that was not contaminated with *Enterobacteriaceae* Lactose Fermenter bacteria producing ESBL was tahu isi (stuffed tofu). One of the factors that can influence this is the use of formalin in tofu. There are still many cases of the use of formalin in tofu sold in markets in various regions in Indonesia. Research conducted by Khaira (2016) showed the presence of formalin in all tofu samples sold at Batusangkar Market. Other research was proven by Rahmawati (2022) who also showed the presence of formaldehyde in all tofu samples sold in several markets in the city of Brebes, Central Java.

Tofu contains protein and high water content, so tofu is easily damaged (Ma'ruf et al, 2017). One day after production, the tofu usually starts to get slimy and smells sour, which is a sign that the tofu is spoiled. By soaking the tofu in formalin, the consistency of the tofu will become denser so that the tofu doesn't crumble easily. In addition, bacteria or other microorganisms cannot grow, so tofu can last up to 7 days (Afriani et al, 2022). The aldehyde element contained in formalin can react with the protein so that it can bind to the protein in tofu both on the outside and inside of the tofu. This causes tofu to become denser and bacteria cannot grow and are unable to produce acid because the protein elements in tofu die from formalin (Afriani et al. 2022).

A total of 19 samples of snack food that were detected to contain *Enterobacteriaceae* Lactose Fermenter bacteria were subjected to the DDST test and the results showed that 13 of them were capable of producing the Extended Spectrum Beta Lactamase (ESBL) enzyme. This research is in line with research by Giri et al (2019) in India, which showed that out of 100 samples of ready-to-eat food on the roadside, 59 bacterial isolates from the *Enterobacteriaceae* group were found, and 16 of them were able to produce ESBL enzymes. Research conducted in Indonesia, precisely in the city of Pontianak, showed the presence of *Enterobacteriaceae* bacteria contamination in 8 samples of cuttlefish snacks sold on the main road (Darna et al. 2018). Another study was conducted by Prasetya et al (2019), that is, out of a total of 30 samples, 8 samples detected *Escherichia coli* bacteria and 4 of them were able to produce ESBL. ESBL enzymes can hydrolyze third and fourth-generation beta-lactam antibiotics, also monobactams (aztreonam) thereby causing multi-resistance to antibiotics (Prasetya 2017). MDR (Multidrug-resistant) is a serious problem faced worldwide because it can lead to reduced therapy options, increased mortality, and length of stay in hospital (Colosi et al. 2020).

CONCLUSION

Enterobacteriaceae Lactose Fermenter contaminants producing Extended Spectrum Beta Lactamase (ESBL) in snack foods sold on the roadside in the Dukuh Kupang market area, Surabaya, were 13 samples (26%). The most contaminated type of snack food is the pohong (cassava), while the non-contaminated snack food is tahu isi (stuffed tofu).

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