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Bacterial Contamination of *Escherichia coli* in Long Beans at Traditional Markets

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Abstract

Escherichia coli (*E. coli*) bacteria may contaminating uncooked food and cause food poisoning disease. Long beans (*Vigna sinensis L*) is one of the vegetable that consumed by people in raw (uncooked) and often traded in traditional markets. Many traditional markets do not meet sanitation requirements and many sellers do not perform good personal hygiene. These situation can potentially to cause bacterial contamination of vegetables sold in traditional markets. There has been no research examining *E. coli* contamination in long beans that sold at traditional markets in Jember.The purpose of this study to prove the contamination of *E. coli* bacteria in long beans at traditional markets in Jember. The research method uses an observational descriptive design with the cross sectional approach. Research places in eight traditional markets in Jember Regency and Microbiology Laboratory, Faculty of Medicine, University of Jember. The samples used amounted to 40 long beans purchased from 40 traditional market traders. The microbiological examination is performed using the most probable number (MPN) method, namely presumptive test and completed test. The results showed from the presumptive test all of 40 samples (100%) contaminated with coliform bacteria. After completed tests, 36 out of 40 samples (90%) were found contaminated by *E. coli.* The majority of long beans sold in traditional markets have been contaminated by *E. coli* bacteria.

Keywords: Long Beans; *E. Coli*; Contamination

INTRODUCTION

Foodborne disease is still a health problem in the world because it is associated with morbidity and mortality of the population. Some 600 million people worldwide have morbidity and 420,000 cases cause mortality per year (Li et al., 2018). Foodborne disease is defined as an infectious or non-infectious disease, acute or subacute, due to contamination of biological or chemical agents that enter the human body through food. The World Health Organization (WHO) estimates there are 31 types of biological agents, including bacteria (66%), viruses (4%), and parasites (4%). The contamination process can occur during production, distribution, and consumption, due to pollution of water, soil, water, storage process, food processing, as well as personal hygiene and low environmental sanitation (Addis & Sisay, 2015).

The incidence of foodborne disease in Indonesia was dominated by home-made food (46%) which is contaminated by pathogenic bacteria (Arisanti et al., 2018). *E. coli* was the bacteria that most often cause outbreaks of foodborne disease (Arisanti et al., 2018). The

presence of *E. coli* bacteria in drinking water or food was an indicator of the fecal material contamination that can endanger public health.

Vegetables eaten raw can cause foodborne disease, as they contain pathogenic bacteria, such as *Listeria monocytogenes, Yersinia enterocolitica, Clostridium perfringens, Bacillus antracis, Salmonella spp., Klebsiella spp.,* and *Escherichia coli* (Syifaa et al., 2019). One of the vegetables that consumed in raw is long beans (*Vigna sinensis L.)*. It is cheap, easy to get, easy to process, and contains proteins and minerals (Reswari et al., 2019). Contamination of *E. coli* in food, when eaten by consumers can cause symptoms of gastroenteritis, abdominal infections, bacteremia, and meningitis (Murray et al., 2016).

People who living in rural areas, especially farming families, prefer to consume traditional foods that containing vegetables harvested from their own gardens (Simanjuntak et al., 2020). On the other side, people who living in urban areas are easier to get fast food that contains high fat (Rahayuningsih & Lestari, 2018). People in urban areas to get vegetables can shop in traditional or modern markets.

Traditional markets in Indonesia are still an important places for majority people to find of vegetables (Iskandar et al., 2018). The price of vegetables was cheaper in the traditional markets than in modern markets (Nelwan & Rumokoy, 2017). There are many traditional markets that facilities still do not meet environmental sanitation and hygiene requirements (Gusti & Sari, 2020). Besides that, there are also many sellers who do not perform personal hygiene properly (Hasana, 2018). These situation can potentially to cause bacterial contamination of vegetables sold at traditional markets. There has been no research examining *E. coli* contamination in long beans that sold at traditional markets in Jember.

Therefore, more research is needed to evaluate the contamination of *E. coli* bacteria in long beans in traditional markets, considering that the traditional market is still the main focus in the process of distributing long beans in the community. The purpose of this study is to prove the *E. coli* contamination in long beans, as well as to know the characteristics of long bean sellers in traditional markets.

METHODS

An observational descriptive study with a cross-sectional approach was conducted. The ethical clearance was approved by Ethical Commission in Faculty of Medicine, University of Jember (No. 1500/H25.1.11/KE/2021). The research was conducted in the Microbiology Laboratory, Faculty of Medicine, University of Jember. Eight traditional markets located in Jember Regency was choosen in this study, including market of Tanjung, Gebang, Kepatihan, Mangli, Arjasa, Sabtuan, Pelita, and Kreongan. The study was conducted in January to February 2021. The research population includes all long beans sold in eight traditional markets and the samples are 40 with purposive sampling techniques. The inclusion criteria used are still fresh and flawless long beans.

The Most Probable Number (MPN) method series 333 was used for microbiology testing. MPN method used, namely presumptive test and completed tests. The sample used as much as 25 grams of long beans dissolved in 225 mL sterile aqua distillation (aquades) and shaken for 15 minutes. Long bean rinse water from each sample (10mL; 1mL and 0.1 mL) in each volume then inoculated in a tube containing 10 mL liquid lactose broth media, three times replication respectively

(tube series 3-3-3). Then incubated at 37 °C (24-48 hours) and 44°C (24 hours). The number of positive tubes after 37°C incubation from each sample was converted into quantitative data using the MPN 333 table according to the Thomas formula. While the positive tubes after incubation at 44°C then carried to completed test. Completed tests by streaking inoculation techniques in *Eosin Methylene Blue* (EMB) Agar media and incubated for 18-24 hours. The positive result of the metallic green sheen colony continued microscopic examination of Gram's staining observed in 1000x magnification microscope.

RESULTS

Forty samples of long beans were purchased from 40 long bean sellers. Characteristics of long bean sellers were include of gender, age, education, duration of selling, as well as the location of selling obtained by conducting interviews (as seen in table 1).

Table 1. Characteristics of long bean sellers

Characteristics	Frequency	
	N	%
Gender		
Male	17	42.5
Female	23	57.5
Age		
Teen (12 - 25 years old)	2	5.0
Adult (26 - 45 years old)	21	52.5
Elderly (46-65 years old)	15	37.5
Seniors (>65 years old)	2	5
Education		
Uneducated	7	17.5
Elementary school	20	50
Junior High School	6	15
Senior High School	7	17.5
Selling location		
Inside the market	28	70
Outside the market	12	30
Selling duration		
Short time (≤5 Hours)	14	35
Long time (>5 Hours)	26	65

Microbiological examination of long beans with MPN method at the stage of the presumptive test showed 26 out of 40 samples (65%) tested at 37 °C for 24 hours were contaminated by coliform bacteria more than 1898 MPN/100 mL (Figure 1) and the rest had varying amounts. The long bean rinse water sample tested presumptively at 37 °C for 48 hours showed all of 40 samples were contaminated by coliform bacteria over 1898 MPN/100 mL, as well as samples tested at 44°C for 24 hours. The total calculation of coliform is shown in Table 2.

Table 2. The results of presumptive test

MPN Index (MPN/100 mL)	Frequency	
	N	%
(37 °C for 24 hours)		
4	1	2.5
7	1	2.5
27	1	2.5
29	2	5.0
58	1	2.5
72	1	2.5
76	1	2.5
95	1	2.5
139	1	2.5
271	1	2.5
438	3	7.5
>1898	26	65
(37 °C for 48 hours)		
>1898	40	100
(44 °C for 24 hours)		

>1898	40	100

The positive results from the samples which incubated at a temperature of 44 °C was suspected contain fecal coliform bacteria that are thermo-tolerant, then conducted completed tests using EMB agar media. Completed test results in Table 3, showing 36 out of 40 samples (90.0%) were contained metallic green sheen colonies of *E. coli* bacteria. All of 36 samples were conducted microscopic examination of Gram's staining, all confirmed Gram negative bacteria form of cocobacilli at a microscope magnification of 1000x (Figure 2).

Table 3. The result of completed test in EMB Agar

Metallic green sheen colonies	Frequency (n)	%
Positive (*E. coli*)	36	90
Negative	4	10

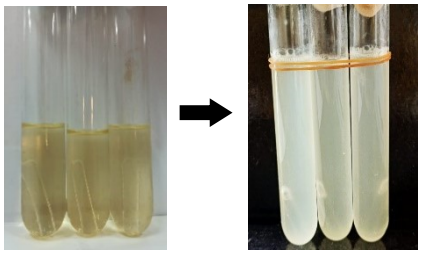
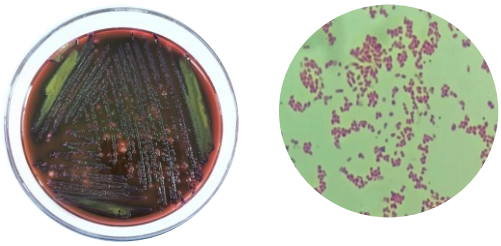


Figure 1. Examination of the presumptive test



(a) (b)

Figure 2. (a) Metallic green sheen on the EMB agar media, (b) Gram staining of *E. coli* bacteria.

DISCUSSION

Majority (57.5%) of the long bean seller were females. Female domination because females are more skilled and easy to socialize (Djangaopa et al., 2018). According to Salamandane et al. (2020), the role of the female in trade is believed to have good hygiene and sanitation practices, thus giving customer confidence in the safety of food sold. The majority (82.5%) long beans trader have poor education (junior high school education or less). Based on age distribution shows most traders are adults (52.5%). This happens because adulthood has high productivity and mobility, balanced by increased economic needs (Purnama & Subrata, 2019). Reviewed based on the place and duration of selling, as many as 28 traders choose to sell in the area within market. However, 12 traders chose to sell outside the market. The average traders has varying sales duration, 26 traders have a selling duration more than five hours, while 14 traders less than five hours. Long selling duration causes vegetables to wither easily due to exposure to room temperature for a long time or direct sun heat, thus affecting the quality of long beans. In addition, the increased activity of food handlers, both biological and chemical pollution allows for a decrease in the quality and safety of vegetables (Pudjirahayu, 2017).

E. coli bacteria are the most dominant gastrointestinal bacteria compared to other enteric bacteria, because its presence per gram feces reaches 10^7-10^9 in humans and 10^4-10^6 in animals (Jang et al., 2017). Food contaminated with *E. coli* bacteria shows that the food has been contaminated with feces. *E. coli* bacterial contamination in long beans can be caused by a variety of factors, ranging from planting, harvesting, post-harvesting, distribution, and processing by consumers. Long

bean contamination in the planting process can be caused by soil contamination, irrigation water, organic fertilizer used, and farmer activities in the garden (Ramadhani et al., 2016). Soil is a natural reservoir of the growth of microorganisms, including *E. coli* bacteria. *E. coli* contamination in fields or plantations due to the defecation activities of animals in the rice fields, the fertilization process by using organic fertilizer derived from animal feces, as well as the disposal of household waste in irrigation around the fields (Luna-Guevara et al., 2019, Le et al., 2014).

Organic fertilizer used in the process of planting long beans serves to improve soil structure, to increase soil pH, and C-organic levels such as nitrogen, phosphorus, potassium, and other plant microelements. The use of organic fertilizer is expected to improve the quality of long bean products compared to inorganic fertilizers. In addition, farmers choose organic fertilizer because the price of organic fertilizer is cheaper and very easy to get (Imran et al., 2017). Although organic fertilizer is often used by farmers, but farmer's knowledge about the dangers of *E. coli* contamination due to the use of organic fertilizer is still limited and often overlooked, because they think improving the quality of long bean products to get a high selling value takes precedence than the safety of the vegetables produced. In organic fertilizers contain nutrients, temperature, humidity, and pH that support for the growth of *E. coli* bacteria. In addition, *E. coli* O157:H7 bacteria in particular have the ability to survive in the soil for 7-25 weeks, meaning it can survive from the beginning of planting to harvesting depending on the type of soil, humidity and environmental temperature of fields (Luna-Guevara et al., 2019).

Improper household waste disposal processes, such as draining kitchen, laundry wastewater, and/or bathroom wastewater into sewers that lead to rice paddy irrigation channels, are certainly factors that affect water and soil contamination. Most farmers in Indonesia directly use field irrigation to provide water supply to long bean crops, but some of them also water long bean crops manually by taking water in rivers or soil wells. Groundwater can be contaminated with *E. coli* due to the distance of soil wells with septic tank infiltration wells of less than ten meters (Salamandane et al., 2020). Although long beans are horticultural plants that hang or do not directly intersect with the soil, *E. coli* bacteria can penetrate the physical protection of plants, such as wax coatings, cuticles, cell walls, trichome, and stomata. This statement is supported by research conducted by Luna-Guevara et al. (2019), which proves stomata can be an entry point for *E. coli* bacteria to penetrate through the leaves. In addition, there is an apoplast which is an extracellular channel of plant cell walls and serves to transport nutrients and water from the roots and xylem. The contamination of water and soil sources at the roots of long bean plants carries a colony of bacteria in the apoplast flow to all parts of the long bean plant, especially the fruit part, due to the properties of *E. coli* bacteria that can grow in aerobic environments, low temperatures, low pH, high UV, and antimicrobial metabolites (Luna-Guevara et al., 2019).

E. coli contamination can occur in the harvesting process. Contamination process at the stage of harvesting long beans occurs when harvesting by hand directly without the use of clean gloves (repeated use of gloves), or do not using sterile tools to pick long beans. Post-harvest storage is only placed on a sack or basket that directly intersects with the soil, without the packaging being covered. If the long beans was not washed after

harvested the residual planting was still carried until the distribution process by distributors.

However, some distributors watering their long beans before arriving at the traditional market to make the long beans sold look fresh. In addition, long beans are also exposed to the surrounding air because of the absence of closed packaging and potential damage to long beans, such as long beans pinched or bent so as to accelerate decay. Distribution from farmers to traders, traders to consumers in traditional markets will affect the quality of long beans (Salamandane et al., 2020, Francis et al., 2018).

Traditional market facilities including clean water access, sanitation, proper packaging and good trader hygiene, will minimize contamination of the long bean. *E. coli* contamination from the process of planting, harvesting, and distribution will continue until the long beans reach the hands of consumers. Proper and correct handling and processing of long beans, after being purchased from the traditional market cutting the chain of contamination of *E. coli* (Francis et al., 2018).

Indonesian food and drug administration explained that the maximum limit of *E. coli* bacterial contamination in ready to eat vegetables is 3 MPN/gram (solid sample) or comparable to 3 MPN/100 mL (liquid sample) (BPOM, 2019). Therefore, long beans sold in traditional markets are not suitable for consumption directly, because they contain fecal coliform bacteria exceeding the maximum limit (more than 1898 MPN/100 mL) and 36 samples are positively contaminated by *E. coli.*

To reduce contamination of *E. coli* in long beans, the following effort need to be made: treatment of long beans in fields using clean water, use of fertilizers containing animal and human waste should be avoided, washing of long beans after harvest, good packaging, keeping cleanliness during the transportation process, improvement of environmental sanitation for selling places in traditional markets and improvement of personal hygiene for vegetable sellers.

CONCLUSION

Long bean sellers is dominated by female, adult age and low educated people. Majority of the sellers was located inside the market, and the duration of selling more than five hours. Ninety percent of long bean samples were contaminated by *E. coli* bacteria. Systematics and continuous effort was needed to avoid contamination of long beans by *E. coli*. From production stage in fields, transportation, distribution and handling by vendors. There is strong suggestion for the consumers that long beans need to be washed by flow water and cooked before eaten.

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