Jurnal Saintika Medika
Jurnal Ilmu Kesehatan dan Kedokteran Keluarga
Faculty of Medicine UMM

Vol. 19 No. 2 December 2023
p-ISSN : 0216-759X e-ISSN : 2614-X476X
http://ejournal.umm.ac.id/index.php/sainmed

Contamination of Coliform Bacteria on Ocimum basilicum at Traditional Markets, Jember Regency

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Received : August 1th2023. Revised : September 24th2023. Published: December 30th2023

DOI: https://doi.org/10.22219/sm.Vol19.SMUMM2.23295

ABSTRACT

Basil is one type of vegetable that can be consumed without any processing. Unprocessed vegetables can cause contamination from Coliform bacteria. This study aimed to determine the number and identification of coliform fecal bacteria as a biological indicator suitable for consumption.This study used descriptive observational with a cross-sectional from November 2020 to March 2021. The research locations are from 8 Traditional Markets in Jember and the Microbiology Laboratory, Faculty of Medicine Jember University. The sample in this study consisted of 40 samples from basil vegetable sellers. The Most Probable Number method was carried out in two stages in this study. First, a presumptive test using lactose broth, and two is, a complete test using Eosin Methylene Blue.The results of the presumptive test obtained 100% of the number of MPNs that exceeded the maximum limit for food quality suitable for consumption according to BPOM. The complete test results showed that 82.5% of basil was contaminated with fecal coliform bacteria, namely Escherichia coli. Practically all the samples were highly infected with coliform bacteria. Suggestions the community is expected to pay attention to techniques in washing and processing basil vegetables properly to break the chain of bacterial contamination.

Keywords : Basil, Coliform, contamination, foodborne diseases

INTRODUCTION

Foodborne disease is an infectious or toxic disease that enters the body due to food contamination by bacteria, parasites, and other toxic compounds (Kim et al., 2018). Most cases of foodborne disease are caused by bacteria. Symptoms of the disease are dominated by gastrointestinal disorders such as vomiting, nausea, abdominal pain, and diarrhea. Previous research by Kirck states, more than 500 million patients experience foodborne disease (Kirk et al., 2015). Research from the Ministry of Health of the Republic of Indonesia stated that the incidence of foodborne disease reached 163 times in one year and the total cases reached 7,132 patients (KEMENKES, 2018).

Vegetables are food ingredients that can be a source of foodborne disease transmission, especially in ready-to-eat foods such as fresh vegetables. Basil is a type of vegetable in fresh vegetables that is often consumed in Indonesia and is served raw. Bunsal Tindry's research on the identification of *Escherichia coli* (*E. coli*) in basil stated that it was found that all positive basil samples contained *E. coli* with levels exceeding the maximum limit according to the Food and Drug Supervisory Agency or BPOM (Bunsal Tindry., 2015). This is a threat to society because the food we consume is not safe and contradicts the PERPU RI number 28 of 2004 article 1 no 7 concerning food quality and nutrition. The PERPU essentially states that food safety is an effort to prevent food from being polluted biologically, chemically, and otherwise that could potentially harm and endanger health.

Resistant bacteria originating from plants, animals, and the environment can colonize the human microbiota through exposure to and/or consumption of contaminated foods. They can also transfer their resistance genes to human intestinal tract pathogens, posing a threat to an effective public health response to the persistent threat posed by infectious diseases (Sun et al., 2021). On the other hand, point-source *E. Coli* epidemics have been linked to animal contact, handling or ingestion of tainted raw or undercooked food, and exposure to faecal contamination in drinking water wells (Mottola et al., 2021). It has been proposed recently that human-pet contact is another possible cause of infection. Although the existence of in basil is recognized, there is currently a dearth of information regarding its incidence in vegetables. Currently, there are not many studies that discuss bacterial contamination in basil vegetables. So, the purpose of this study was to determine the number of coliform bacteria in basil isolates that had met the requirements of biological indicators for the feasibility of food quality that was safe for consumption and, to determine the presence of *E. coli* bacterial contamination in basil isolates sold in the Jember traditional market.

METHODS

This data collection has received approval from the Ethics Commission of the Faculty of Medicine, University of Jember with letter number 1432/H25.1.11/KE/2020. The research design used is descriptive observational with a cross-sectional approach. The design of this study can describe the characteristics of traders and the contamination of E. coli bacteria in basil vegetables that are sold at one time. The research was carried out in 8 traditional markets and the Microbiology Laboratory, Faculty of Medicine, University of Jember. The 8 traditional markets used in the study came from Jember Regency which included Tanjung Market, Gebang Market, Kepatihan Market, Mangli Market, Arjasa Market, Saturday Market, Pelita Market, and Kreongan Market. The duration of the study was from November 2020 to March 2021. The study population included all basil sold in eight traditional markets. The research sample used was 40 samples with purposive sampling technique. The inclusion criteria used were basil which was still fresh and not rotten and deformed.

Microbiological analysis in this study used the Most Probable Number method or MPN series 333. The MPN method used consisted of two tests, namely a presumptive test using lactose broth

media and a complete test using Eosin Methylene Blue (EMB) media. The sample used in this study was 25 grams of basil leaves and dissolved in 225 mL of sterile distilled water in an erlenmeyer. Then the sample was shaken for 15 minutes. Aquades that have been shaken are called basil rinse water which is then inoculated in lactose broth medium in a ratio of 1:1, 1:10〗^(-1), 〖1:10〗^(-2). The media was then incubated at 37 °C for 48 hours and at 44 °C for 24 hours. A positive result is indicated by the media turning cloudy and producing gas in the Durham tube.

A positive result on lactose broth media was then followed by a complementary test using the media. The technique used for this medium was striking using a round loop. A positive result of *E. coli* bacteria will be identified by the color of the metallic sheen/green colony.

RESULTS AND DISCUSSION

The results of the presumptive test showed that 100% of the MPN samples at 37°C were positive for coliform bacteria with an amount of 1898 MPN/100 ml. Meanwhile, the results of the basil samples at 44 °C were positive for coliform bacteria containing 1898 MPN/100 ml as much as 97.5% and 2.5% containing 271 MPN/100 ml coliform bacteria (see fig. 1 and table 1).

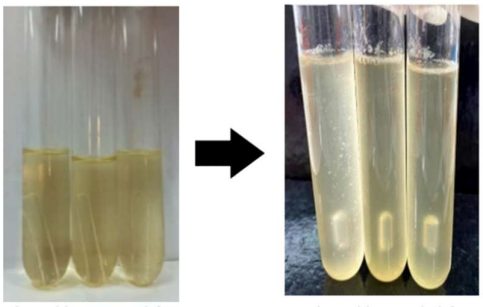

Figure 1. Result of MPN test

Table 1. The result of *Coliform* total count in MPN test

Incubation	MPN Index	(n:40)	
	MPN/100 ml	n	%
37C 48 H	≥1898	40	100.00%
44C 24 H	≥1898	39	97.50%
	≥271	1	2.50%
Not Eligible to consumption > 3 MPN/100 ml			

This shows that the MPN value of coliform bacteria in basil samples in 8 traditional markets in Jember Regency does not meet the requirements for the maximum threshold for coliform bacterial contamination for ready-to-eat vegetables according to BPOM because the amount is >3 MPN MPN/100ml.

This shows that basil in all samples exceeds the maximum limit as an indicator of the feasibility of ready-to-eat food which should be <3 MPN/100ml. Similar findings to these results was also reported in many other previous studies. Study conducted by Waturangi et al. in Jakarta which stated that 98.4% of samples of vegetables such as carrots, lettuce, basil did not meet the requirements for food quality that were safe for direct consumption (Waturangi et al., 2019). This study was also supported by Sari who stated that 100% of the samples from the MPN examination on basil in Pontianak City did not meet the eligibility requirements for food quality that was safe for direct consumption, or the amount was >3 MPN/100 ml (Sari et al., 2019). Another study by Hassan et al. (Hassan and Purwani, 2016) found that the level of microbial contamination was relatively high, particularly in the Thai Lemon Basil in West Java, where the TPC was >9 log10 cfu/g. The observations made by Attien et al.(Attien et al., 2020) when researching the social network of market gardeners in Abidjan are consistent with this. In Singapore (Li and Uyttendaele, 2018), Cameroon (Akoachere et al., 2018), Canada (Denis et al., 2016), and Ghana (Adzitey, 2018) similar observations demonstrating the involvement of wastewater in the contamination of vegetables produced on market garden production sites have been documented.

The results of the complete test showed that there were 82.5% positive samples of *E. coli* (table 1). These positive bacterial colonies can be identified easily because they have a distinctive color of the colony, namely metallic sheen/green (fig. 2 and table 2).

Figure 2. Result of methalic sheen colony in EMB Media cultur

Table 2. The prevalence of *E. coli* isolated from *Ocimum basilicum*

Market	N	Positive	Percentage
KP	5	5	12.50%
TJ	5	5	12.50%
KR	5	4	10.00%
G	5	3	7.50%
PE	5	5	12.50%
A	5	3	7.50%
M	5	3	7.50%
S	5	5	12.50%
Total	40	33	82.50%

KP: Kepatihan, TJ: Tanjung, KR: Kreongan, G: Gebang, PE: Pelita, A: Arjasa, M: Mangli, S: Sabtuan

The results of research samples that have been tested on EMB media show that 82.5% of the samples identified *E. coli*. The results in this study are in line with Bunsal Tindry which stated that *E. coli* in basil found in 8 food stalls in Manado City was 100% positive for *E. coli* (Bunsal Tindry., 2015). Other vegetable contamination such as lettuce in California plantations also stated that 96.7% of samples were contaminated with E. Coli (Jeamsripong et al., 2019), 33.3% in Germany (Becker et al., 2019), 28.2% in Korea (Kapeleka et al., 2020), 32% in Nepal (Ghimire et al., 2020).

The wide variation in prevalence could be caused by a variety of elements. These could include things like geographic location, the kind and quantity of samples studied, the techniques used to find bacteria, the kind of water used for irrigation, (Rahman et al., 2022) and the post-harvest handling techniques for these veggies, which vary from country to country (Ghimire et al., 2020)

Contamination of *E. coli* in vegetables can be caused by various factors and stages such as planting, harvesting, and processing. Vegetable contamination in the planting process is caused by contamination from soil, water used for irrigation, fertilizers, and farmer activities. Contamination of *E. coli* in soil can occur due to several factors, such as defecation from livestock and wild animals in plantations, and the use of organic fertilizers derived from animal waste, namely cows, goats, chickens, etc (Allende et al., 2017; Luna-Guevara et al., 2019). Water for irrigation on vegetables that are careless can also be a route for contamination of *E. coli*, one of which is the use of river water which is still often used as a place for garbage disposal and excreta disposal (O'Flaherty et al., 2019).

The harvesting process can also cause basil to be contaminated by *E. coli*. This can happen because in the harvest process, farmers do not wear clean gloves to avoid bacterial contamination. The distribution process can also potentially contaminate vegetables. So, vegetables should be packaged, placed in a closed container, and separated from other vegetables (Yuniastri et al., 2018).

Cleanliness or hygine at traders also greatly affects the contamination and proliferation of E. coli bacteria. Merchant hygiene is an effort to maintain and protect the cleanliness of traders. How to maintain hygiene at traders can be done by applying the following steps, namely washing hands with soap before and after buying and selling transactions with consumers (Bolten et al., 2020), not smoking, using Personal Protective Equipment (PPE) such as aprons, masks, head coverings etc., checking health every time. month to monitor the health of traders and prevent infectious diseases (Efendi and Syifa, 2019).

The use of aprons on traders serves to protect traders from contaminating their merchandise and not soiling their clothes. The apron to be used must be clean and should not be used for wiping hands or tables. Hair should also be covered as a form of neatness, aesthetics. and prevention of food contamination by pathogenic bacteria. The use of masks when trading is also considered to be able to avoid bacterial contamination of food. Wash hands before and after activities and keep fingernails clean and cut short. Waste management must also be good because waste that is not managed properly can be a factor causing foodborne disease. This can happen because the trash can is a nesting place for flies. Flies are vectors that can transfer disease agents by contaminating the food they infest by moving bacteria on the surface of their bodies. These animals can carry pathogenic diseases such as *E. coli*, *Salmonella enterica*, and *Listeria monocytogenes* without experiencing physiological disturbances in their bodies (Andiarsa, 2018). Therefore, it is necessary to do good waste management for traders, use repellents or fly traps as an effort to prevent bacterial contamination by vectors. Proper handling and management of vegetables is needed to break the chain of contamination caused by bacteria. Proper handling and processing of vegetables, once purchased from traditional markets can also cut the chain of contamination in food (Akoachere et al., 2018)

CONCLUSION

This study showed that most of the basil isolates sold in 8 traditional markets in Jember Regency were contaminated with *E. coli* and did not meet the food quality requirements that were safe for direct consumption. It is necessary to pay more attention to seller's hygiene to reduce the occurrence of bacterial contamination in basil and consumers are expected to pay attention to techniques in washing and processing vegetables properly to break the chain of bacterial contamination.

In addition, the molecular genotyping of *E. coli* and the identification of particular virulence factors underscore the necessity of initiating an inventive surveillance system grounded in biomolecular characterization for an integrated microbiological risk assessment of basil. This will enable the implementation of inventive supply chain control, ultimately ensuring the safety of consumers. In light of this, microbiological risk assessment of *E. coli* in the supply chain and an inventive food-management system require molecular approaches that enable the quick and precise quantification, identification, and characterization of *E. coli* in foods.

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