The Correlation Between Food Hygiene and Sanitation in Food Vendors of *Lalapan* with *Enterobacteriaceae* Contamination in Fresh Vegetables

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Tenaga Pengajar Mikrobiologi Kedokteran

Fakultas Kedokteran Universitas Jember

KEMENTRIAN PENDIDIKAN, KEBUDAYAAN, RISET DAN TEKNOLOGI

UNIVERSITAS JEMBER

Karya Ilmiah di publikasikan pada:

Jurnal Ilmiah Kesehatan (*Journal of Health Science*)

Vol. 16 No.1 (Maret 2023)

Universitas Nahdatul Ulama Surabaya

ISSN 1978-6743

e- ISSN 2477-3948

ISSN 1978-6743
E-ISSN 2477-3948

UNUSA

SK. No. 85/M/KPT/2020
S4
ACCREDITATED

Jurnal Ilmiah Kesehatan

(Journal of Health Science)

Fakultas Keperawatan dan Kebidanan
Universitas Nahdlatul Ulama Surabaya


SERTIFIKAT

Kementerian Riset dan Teknologi/
Badan Riset dan Inovasi Nasional

RISTEK-BRIN

Petikan dari Keputusan Menteri Riset dan Teknologi/
Kepala Badan Riset dan Inovasi Nasional
Nomor 85/M/KPT/2020
Peringkat Akreditasi Jurnal Ilmiah Periode I Tahun 2020
Nama Jurnal Ilmiah
Jurnal Ilmiah Kesehatan (Journal of Health Sciences)
E-ISSN: 24773948
Penerbit: Universitas Nahdlatul Ulama Surabaya

Ditetapkan sebagai Jurnal Ilmiah
TERAKREDITASI PERINGKAT 4
Akreditasi Berlaku selama 5 (lima) Tahun, yaitu
Volume 13 Nomor 1 Tahun 2020 sampai Volume 17 Nomor 2 Tahun 2024

Jakarta, 01 April 2020
Menteri Riset dan Teknologi/
Kepala Badan Riset dan Inovasi Nasional
Republik Indonesia,

Bambang P. S. Brodjonegoro

About the Journal

Jurnal Ilmiah Kesehatan (Journal of Health Science) is a peer review scientific journal published by the Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya. JHS establish in 2009, with p-ISSN 1978-6743; e-ISSN 2477-3948. JHS is published periodically thrice a year (February , May and August). jurnal ilmiah kesehatan(journal of health sciences) is intended Provide for dissemination of original and quality research on various topic in nursing, midwifery and health.

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Jurnal Ilmiah Kesehatan
(Journal of Health Science)
Fakultas Keperawatan dan Kebidanan | Universitas Nahdlatul Ulama Surabaya
ISSN 1978-6743
E-ISSN 2477-3948
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(Journal of Health Science)
Fakultas Keperawatan dan Kebidanan | Universitas Nahdlatul Ulama Surabaya
ISSN 1978-6743
E-ISSN 2477-3948
S4 ACCREDITATED

Vol. 16 No. 01 (2023): Jurnal Ilmiah Kesehatan (Journal of Health Science)

DOI: https://doi.org/10.33086/jhs.v16i01

PUBLISHED: 2023-02-28

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DOI : 10.33086/jhs.v16i01.3693
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ISSN 1978-6743
E-ISSN 2477-3948
Jurnal Ilmiah Kesehatan
(Journal of Health Science)
Fakultas Keperawatan dan Kebidanan | Universitas Nahdlatul Ulama Surabaya

The Correlation Between Food Hygiene and Sanitation in Food Vendors of *Lalapan* with *Enterobacteriaceae* Contamination in Fresh Vegetables

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ARTICLE INFORMATION

Received: June 7, 2022
Revised: February 21, 2023
Available online: February 2023

KEYWORDS

Food hygiene and sanitation; Food vendors; Enterobacteriaceae contamination; Fresh vegetables

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A B S T R A C T

Lalapan is a fresh vegetable usually eat up with rice and other Indonesian dishes. However, fresh vegetables can potentially be contaminated with pathogenic bacteria, such as *Enterobacteriaceae*, harming consumers if not appropriately processed and cleaned. This study analyzes the correlation between food hygiene and sanitation in food vendors of *Lalapan* with *Enterobacteriaceae* contamination in fresh vegetables. It used an analytic observational design with a cross-sectional approach. In addition, the population was food vendors of *lalapan* in the Sumbersari District, Jember Regency. There were 30 respondents with a consecutive sampling method. Food hygiene and sanitation instrument was an observation sheet according to 16 points stipulated in the Regulation of the Minister of Health of the Republic of Indonesia number 1096/MENKES/PER/VI of 2011. In addition, we tested fresh vegetable samples to analyze *Enterobacteriaceae* contamination with a microbiological test using Salmonella Chromogenic Agar (SCA) media. Then, data analysis used Fisher's exact test with α=0.05. The observation showed that most respondents had good food sanitation hygiene (76.7%). The microbiological examination indicated 23 vegetable samples (76.7%) were contaminated with *Enterobacteriaceae*. Statistical analysis using Fisher's exact test Exact obtained p=1.000 (p>0.05). Thus, there was no significant correlation between food hygiene and sanitation in food vendors of *lalapan* with *Enterobacteriaceae* contamination in fresh vegetables. In conclusion, hygiene, and sanitation food among food vendors of *lalapan* do not correlate with *Enterobacteriaceae* contamination in fresh vegetables. Further research could analyze the vegetable's planting, harvesting, and distribution processes as risk factors for Enterobacteriaceae contamination in fresh vegetables.

INTRODUCTION

Indonesian people, especially in Java, widely consume *Lalapan*. *Lalapan* is a fresh vegetable usually eat up with rice and other Indonesian dishes. It is in great demand because it is affordable, practical, and has many benefits. Nutrients from fresh vegetables do not change due to no processing or raw presentation. However, fresh vegetables can potentially be contaminated with pathogenic bacteria, harming consumers if not appropriately processed and cleaned (Purba, Chahaya, and Marsaulina, 2014).

The characteristics of safe food are fresh ingredients, good quality, and the absence of contamination from pathogens or harmful substances (Badan Pengawas Obat dan Makanan, 2015). Unmet requirements for safe food can occur health problems, especially foodborne diseases. Foodborne disease is a disease occurring due to food contamination by contaminating agents such as bacteria (66%), chemicals (26%), viruses (4%), and parasites (4%) (Al-Seghayer and Al-Sarraj, 2021). Several bacteria from members of the

https://doi.org/10.33086/jhs.v16.i01.3053

Enterobacteriaceae family are pathogens that often contaminate food and beverages. *Enterobacteriaceae* bacteria, which are pathogenic, including the genus *Salmonella*, *Shigella, and Escherichia*, can cause diarrhea and diseases outside the digestive tract (Woh *et al.*, 2017).

Unsafe food is a crucial global public health issue (Sari, Marsaulina, and Chahaya, 2013). Over 200 diseases and 420,000 deaths are caused yearly by consuming food contaminated with bacteria, viruses, parasites, and harmful chemicals (World Health Organization, 2022). The Indonesian Basic Health Research (2018) found an increased diarrhea incidence in all age groups, from 4,274,790 in 2017 to 4,504,524 in 2018. *Salmonella* species contribute to 25 million incidences of foodborne disease, with around 200,000 deaths annually globally. The number of cases caused by *Shigella* species is estimated at 165 million (Mardu *et al.*, 2020). *E. coli* O157:H7 also causes about 20% of foodborne disease outbreaks (Getaneh *et al.*, 2021).

Several studies showed a correlation between food contamination and poor food processing behavior. Food sellers' knowledge, attitudes, and practices related to food safety affect food contamination. Food vendors have the potential to be the media to spread pathogens causing foodborne diseases. Food vendors not washing their hands can transmit pathogens, especially from fecal-oral routes. Pathogens can also spread through unclean cooking utensils and cutlery. An investigation by Tóth *et al.* (2018) swabbed the cooking utensils and cutlery that come in contact with food. It found the contamination of aerobic mesophilic bacteria in most of those samples. In addition, a study in Jember Regency showed there was contamination of *E. coli* bacteria in 90% of the long bean vegetables sold in traditional markets (Shodikin *et al.*, 2022).

Foodborne diseases such as diarrhea and typhoid fever remain relatively high in JemberDistrict. The diarrhea incidence among the Subdistricts in the Jember Districts was 2,767 in Sumbersari, 2,541 in Kaliwates, 2,496 in Bangsalsari, 2,070 in Patrang, and 1,631 in Ajung. Further, the population density was correlated with many cases in urban areas, such as Sumbersari, Kaliwates, Bangsalsari, and Sumbersari (Dinas Kesehatan Kabupaten Jember, 2015). Several previous studies revealed that food hygiene and sanitation in food vendors were one of the risk factors for foodborne disease. However, no previous studies have examined the correlation between food sanitation and hygiene with *Enterobacteriaceae* contamination on fresh vegetables. This study analyzes the correlation between food hygiene and sanitation in food vendors of *Lalapan* with *Enterobacteriaceae* contamination in fresh vegetables in the Jember Regency.

https://doi.org/10.33086/jhs.v16.i01.3053

METHOD

This research used an analytic observational design with a cross-sectional approach. The population was food vendors of *lalapan* in the Sumbersari Subdistrict, Jember District. In addition, there were 30 respondents with a consecutive sampling method. The study was carried out from November 2021 to April 2022. Before the research, the authors gave informed consent to the respondents. The inclusion criteria were (1) a food vendor of *lalapan* in the Sumbersari Subdistrict, Jember District, (2) respondents selected cooking ingredients, did ingredients storage, ingredients preparation, food processing, food transportation, and food serving, and (3) being willing to be the respondent. The exclusion criteria were food vendors of *lalapan* with incomplete observation data related to food hygiene and sanitation.

The authors directly observed food hygiene and sanitation behavior in food vendors of *lalapan* with observation sheets during food-selling activities without intervention from researchers to avoid bias if the seller knew their actions were being observed. Food hygiene and sanitation instrument was an observation sheet according to 16 points stipulated in the Regulation of the Minister of Health of the Republic of Indonesia number 1096/MENKES/PER/VI of 2011 (Meteri Kesehatan Republik Indonesia, 2011). The instrument was also used in the previous studies conducted by Tarigan (2019), Ismail *et al.* (2016), Khotimah (2015), and Stratev *et al.* (2017). There were 16 items in the observation sheet. Respondents with ≥ eight items' activities on the observation sheet were scored as good, while < eight points were categorized as poor food sanitation hygiene.

In addition, we tested fresh vegetables at the Microbiology Laboratory, Faculty of Medicine, Jember University, to analyze bacterial contamination with a microbiological test using Salmonella Chromogenic Agar (SCA) media. We put vegetable samples into an Erlenmeyer tube with sterile distilled water and homogenized them with a vortex mixer. The liquid fresh vegetable sample that has been homogeneous was taken using an Ose needle, then planted on the SCA media in laminar airflow. After that, the media was incubated at 36-37°C for 24 hours. After 24 hours, we observed the incubation to see the color of the colonies growing on SCA media. Blue-green colonies contained the contamination of *E. coli*, purple colonies were *Salmonella*, and clear/colorless colonies were *Proteus* bacteria. Then, data analysis used Fisher's exact test with α=0.05.

RESULT

Most respondents in this paper were female (83.3%) and adults (76.7%). In addition, almost half of them had low education (Table 1).

https://doi.org/10.33086/jhs.v16.i01.3053

Table 1. The characteristics of respondents by sex, age, and level of education

Characteristics of respondents	Frequency (n)	Percentage (%)
Sex		
Male	5	16.7
Female	25	83.3
Age		
Adolescents (12-25 years old)	4	13.3
Adults (26-59 years old)	23	76.7
Older people (>60 years old)	3	10
The levels of education		
Under senior high school	12	40
Senior high school	10	30.3
University	8	26.6

The food hygiene observation included the stages of cooking ingredients selection, ingredients storage, ingredients preparation, food processing, food transportation, and food serving. In addition, food sanitation assessed the use of closed and clean containers, personal hygiene, and the hygienic handling of food (Table 2).

Table 2 The observation of food hygiene and sanitation in the respondents

Food hygiene and sanitation	Observation			
	Yes		No	
	n	%	n	%
Food vendors kept the food in clean and safe containers for health.	28	93.3	2	6.7
Food vendors stored food in separate containers for each type of food.	24	80	6	20
Food vendors stored the food in closed containers.	6	20	24	80
Food vendors washed fresh vegetables using soap and running water.	19	63.3	11	36.7
Food vendors touched food using gloves.	1	3.33	29	96.7
Food vendors touched food using spoons or ladles.	27	90	3	10
Food packaging was clean and did not contaminate food.	30	100	0	0
Food vendors served food in clean containers.	29	96.7	1	3.3
Food vendors served food in sealed containers.	11	36.7	19	63.3
Food vendors washed the cutlery with running water and soap.	21	70	9	30
Food vendors immediately served the food after the cooking process.	25	83.4	5	16.6
Food vendors wore clean clothes, aprons, or head coverings.	6	20	24	80
Food vendors washed their hands properly every time they touched food.	11	36.7	19	63.3
Food vendors keep nails clean (nails are not long and not dirty)	13	43.3	17	56.7
Food vendors did not use jewelry (rings or bracelets).	18	60	12	40
The food vendor had no wounds or ulcers on his hands.	26	86.6	4	13.4

Furthermore, most respondents had good food sanitation hygiene (76.7%), and only seven respondents (23.3%) were in the poor category (Table 3).

Table 3 Food hygiene and sanitation scoring

Food hygiene and sanitation scoring	Frequency (n)	Percentage (%)
Good	23	76.7
Poor	7	23.3

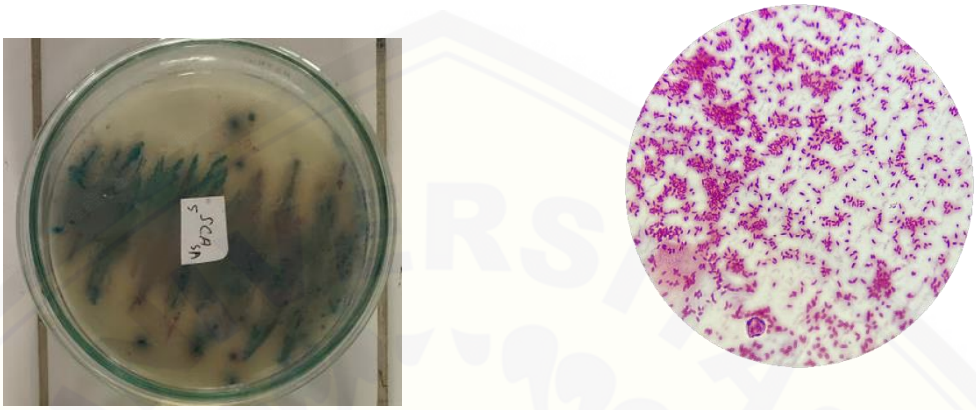
The microbiological test showed that 23 (76.7%) vegetable samples were contaminated with *Enterobacteriaceae*. Furthermore, of the 23 samples contaminated with *Enterobacteriaceae*, 22 were contaminated with *E. coli*, indicating bluish-green colonies on SCA media. In addition, 19 contained

https://doi.org/10.33086/jhs.v16.i01.3053

Salmonella showing purple colonies on the media. Furthermore, three samples found colorless colonies on the media, indicating *Proteus* bacteria contamination.

Table 4 Microbiological tests using Salmonella Chromogenic Agar Media

Microbiological tests using *Salmonella Chromogenic Agar*	Frequency	
	n	%
Contaminated with *Enterobacteriaceae*	23	76.7
Uncontaminated with *Enterobacteriaceae*	7	23.3



(a) (b)

Figure 1. (a) Microbiological test results with SCA media (b) Microscopic image

Of the respondents with good food hygiene and sanitation, 60% had samples contaminated with *Enterobacteriaceae* and 16.7% uncontaminated with *Enterobacteriaceae*. Meanwhile, of respondents with poor food hygiene and sanitation, 16.7% had samples contaminated with *Enterobacteriaceae* and 6.6% uncontaminated with *Enterobacteriaceae*. Statistical analysis using Fisher's exact test Exact obtained p=1.000 (p>0.05). Thus, there was no significant correlation between food hygiene and sanitation in food vendors of *lalapan* with *Enterobacteriaceae* contamination in fresh vegetables (Table 5).

Table 5. The correlation between food hygiene and sanitation with *Enterobacteriaceae contamination*

Variable	*Enterobacteriaceae* contamination				Total		Fisher's Exact Test
	Positive		Negative				
Food hygiene and sanitation	n	%	n	%	n	%	
Good	18	60	5	16.7	23	76.7	p=1.000
Poor	5	16.7	2	6.6	7	23.3	
Total	23	76.7	7	23.3	30	100	

DISCUSSION

There were more female respondents than males in this paper, with a ratio of 5:1. It is because women process food more often and will carry out good food hygiene and sanitation more often than men. Therefore, consumers had more trust in women food sellers regarding food safety (Salamandane *et al.*,

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2020). In addition, a study by Nee and Sani (2011) showed that women had better food hygiene and sanitation than men.

Adult respondents were also more dominant in this research than adolescents and older adults. It is because the age factor can affect a person's work productivity. Adults tend to be more productive and have increasing economic demands. It aligns with Lihu, Warouw, and Akili's study. The study found that adult sellers were the most dominant in all age groups.

Another factor that can influence behavior is the level of education. The results showed that most respondents had a low level of education. Only nine graduated from junior high school, two graduated from elementary school, and one did not attend school. Education is a predisposing factor in changing health behavior in individuals. An education level affects knowledge and attitude that will impact decision-making (Rachmawati, 2019). The higher the education among food sellers, the better food hygiene and sanitation behavior (Aspiani and Rustiawan, 2020).

The author's observations among food vendors of *lalapan* in the Sumbersari Subdistrict showed that most had good food hygiene and sanitation. In the food storing process, most respondents stored the food in clean and separate containers. In addition, all the respondents used clean and safe food packaging. Most also washed fresh vegetables (63,7%) and cutlery (70%) using soap and running water. Fresh vegetables can be contaminated with microorganisms or chemical substances. Therefore, fresh vegetables must be washed properly. Eryando, Susanna, and Kusuma (2014) research also found that most sellers washed food ingredients and cutlery before preparing the food.

However, the behavior of washing hands properly and using running water before and after handling food among respondents still needed improvement. Dirty hands can transfer microorganisms and other harmful materials to food. Therefore, The Ministry of Health of the Republic of Indonesia gives guidance on six steps of washing hands. In addition, the percentage of respondents who did not keep their nails clean was more (56.7%) than respondents who kept their nails clean. It is similar to Rahmadhani and Sumarmi's (2017) research. That research found that most food handlers did not keep their nails clean or had long and dirty nails. Dirty nails and hands can be a source of contaminants or result in cross-contamination.

Most respondents in this paper did not cover food containers to store fresh vegetables, although they kept most foods separately for each type. It can risk the spread of pathogens through vectors such as flies, mice, and cockroaches (Morestavia and Sulistyorini, 2014). Moreover, almost all food vendors did not use gloves when handling food, but most used spoons or ladles. Using utensils in food processing can reduce the risk of contamination in processed food rather than touching it directly by hand. Furthermore, 80% of respondents did not use personal protective equipment such as aprons, head coverings, and masks. Thus, it did not meet the requirements for food handlers according to the Regulation of the Minister of Health of

https://doi.org/10.33086/jhs.v16.i01.3053

the Republic of Indonesia, Number 1096/MENKES/PER/VI of 2011, concerning food hygiene and sanitation (Meteri Kesehatan Republik Indonesia, 2011).

The microbiological test on fresh vegetable samples showed that most were contaminated with *Enterobacteriaceae* (76.7%). Furthermore, Salmonella Chromogenic Agar media showed contaminations of *E. coli* (green-blue colonies), *Salmonella* (purple colonies), and *Proteus* (clear/white colonies) bacteria. Thus, the fresh vegetable samples in this paper did not meet the Regulation of the Minister of Health of the Republic of Indonesia number 1096/MENKES/PER/VI of 2011 concerning Food Hygiene and Sanitation, which states that the microbiological test in food must be 0/gr of food.

Enterobacteriaceae contamination in fresh vegetables can be caused by several factors, starting from the vegetables' planting, harvesting, distribution, and processing stages. *Enterobacteriaceae* are Gram-negative bacteria that are part of the normal flora of the lower digestive tract but can also be found in the environment, such as in soil, water, and object with feces contamination (Ncube *et al.*, 2020). *Enterobacteriaceae* contamination in the planting process can occur due to bacterial contamination of the soil, irrigation water contaminated with sewage, and farmer activities when growing vegetables (Warsyidah, 2017). In addition, vegetable farmers use organic fertilizers made from livestock manure to improve the quality of plantation soil fertility. It is because organic fertilizers contain ingredients that support bacterial growth. When those vegetables are unwashed and eaten raw, microorganisms can enter the body and infect humans (Purba, Chahaya, and Marsaulina, 2014).

In addition, groundwater as a source of field irrigation can also be contaminated by indiscriminate waste disposal, such as household waste being dumped into ditches until it ends up in plantation irrigation. Vegetable farmers can potentially use that contaminated irrigation water, causing *Enterobacteriaceae* contamination in vegetables (Rianti, Buana, and Kiyat, 2018). *Enterobacteriaceae* contamination can also occur during the harvest process. It is due to poor sanitation during harvest, such as not using clean gloves to store vegetables also uncleaned and ground-contact containers. In addition, contamination can occur from uncleaned or incorrectly washed vegetables in the distribution process. Most distributors only water the vegetables with inadequate water to make them look fresh when sold. Thus, good packaging to store vegetables sold in the market will reduce *Enterobacteriaceae* contamination. In addition, markets sanitation where vegetables are sold is also critical.

Clean water meeting the requirements also plays a vital role in food processing. The biological parameters of clean water for sanitation hygiene purposes were guided by the Regulation of the Minister of Health of the Republic of Indonesia Number 32 of 2017 concerning Environmental Health Quality Standards and Water Health Requirements for Sanitary Hygiene, Swimming Pools, Solus Per Aqua, and Public Baths (Menteri Kesehatan Republik Indonesia, 2017). Clean water must have maximum *Coliform* bacteria

https://doi.org/10.33086/jhs.v16.i01.3053

content of 50 CFU /100 ml and 0 CFU/100 ml for *E. coli* bacteria. It is essential in washing materials and equipment, food processing, to food serving. Microorganisms contamination can occur if the water used is not by established health standards. One way to reduce the risk of contamination is to wash raw vegetables in clean, running water. If not washed properly, bacteria will still be present on vegetables when served as fresh vegetables (Eryando, Susanna, and Kusuma, 2014). However, a prior study revealed that food handlers often used water that did not meet the requirements or even did not wash vegetables. Thus, there would be potential fecal transmission through the vegetables served, especially if the vegetables are served raw (Nuryani, Adiputra, and Sudana, 2016).

This research indicated no significant correlation between food hygiene and sanitation in food vendors of *lalapan* with *Enterobacteriaceae* contamination in fresh vegetables (p=1.000). However, previous research showed a significant correlation between food hygiene and sanitation with *Enterobacteriaceae* contamination. A study conducted by Ratna and M. M. Simatupang (2019) indicated a significant correlation between food hygiene and sanitation among food handlers with bacterial contamination in snacks. In addition, there was a relationship between food hygiene and sanitation with *E.coli* contamination in street food with a p=0.015 (Syafriyani and Djaja, 2019).

Thus, this paper showed different results from previous studies. It might be due to the unhygienic behavior of vegetable farmers (from planting to harvesting), vegetable distributors, and sellers. In addition, not using clean water and poor market sanitation are risk factors for Enterobacteriaceae contamination in fresh vegetables. *Enterobacteriaceae* contamination in vegetables indicates fecal transmission. It can occur from the planting stage to the vegetable processing stage.

Several other predisposing factors for bacterial contamination in vegetables are the water quality used to wash vegetables and cutlery, poor environmental conditions, and inadequate knowledge and attitude in food vendors. Using unclean water to clean vegetables and cutlery can cause contamination in fresh vegetables. Bedadung River is a raw water supplier for Local Government-Owned Water Utilities (Perusahaan Daerah Air Minum, PDAM) in Jember Regency, especially in Sumbersari District (Pradana *et al.*, 2019). Unfortunately, 25% of toilet waste produced by residents living around the Bedadung River is disposed of directly into the river (Puspitasari, Novita, and Pradana, 2021). Thus, it can lead to the contamination of river water by *Coliform* bacteria found in human feces, such as *E. coli*. In addition, respondents might use water sourced from dug wells. The well's walls made of soil can cause contamination of water through the soil pores, affecting water quality. Furthermore, the close distance between the septic tank and the water well can pollute the water (Kurniasih, Nurjazuli, and Hanani D., 2015).

https://doi.org/10.33086/jhs.v16.i01.3053

Thus, training on food safety, especially in food hygiene and sanitation, is essential. A study conducted by Nik Husain *et al.* (2016) revealed that food safety training enhanced food handlers' knowledge, attitudes, and practices. So, it can reduce the potential for foodborne disease incidence. In addition, good water management in PDAM and public understanding regarding using water sources to clean vegetables and cutlery can prevent *Enterobacteriaceae* contamination in fresh vegetables.

CONCLUSION

In conclusion, hygiene, and sanitation food among food vendors of *lalapan* do not correlate with *Enterobacteriaceae* contamination in fresh vegetables. Further research could analyze the vegetables' planting, harvesting, and distribution processes as risk factors for *Enterobacteriaceae* contamination in fresh vegetables. Food vendors of *lalapan* and consumers should wash and cook fresh vegetables until they are cooked to be safe for consumption.

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