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Contamination of *Escherichia coli*, *Salmonella sp.* and *Vibrio sp.* on ice cubes at food stalls in Karangasem Regency, Bali Province in 2021



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I Gede Wikania Wira Wiguna¹, Agus Eka Darwinata², Komang Januartha Putra Pinatih²,
Ni Nengah Dwi Fatmawati^{2*}

ABSTRACT

Background: Waterborne diseases spread through drinking water and food products made from drinking water, including cooking utensils that are washed using contaminated water. Ice cube is widely used by the people in Indonesia. There was no study regarding bacteria contamination on an ice cube in Karangasem Regency. This study aimed to determine the contamination of *Escherichia coli*, *Salmonella sp.*, and *Vibrio sp.* on ice cubes at food stalls in Karangasem Regency in 2021.

Methods: This study used a cross-sectional research design and simple purposive sampling as the sampling technique. Ice cubes were taken from 42 food stalls from eight sub-districts. Samples were cultured on Lactose Broth (LB) media for the Most Probable Number (MPN) test procedures and Eosin Methylene Blue Agar (EMBA) to identify the presence of *E. coli*, *Salmonella Shigella*

Agar (SSA) for *Salmonella sp.*, and Thiosulfate Citrate Bile Sucrose (TCBS) for *Vibrio sp.* and followed by gram staining. Data processing was carried out descriptively to determine the percentage of samples with positive contamination. Data were analyzed using Microsoft Excel version 2010 for Windows.

Results: Based on the study results, it was found that all samples contained coliform bacteria through the MPN test (100.0%). In addition, 5 samples (11.9%) were found positive for *E. coli*. While the identification of *Salmonella sp.* and *Vibrio sp.* was found no positive results.

Conclusions: There was coliform bacteria contamination in the tested ice cube samples. In addition, *E. coli* was found in the sample; however, there were no *Salmonella sp.* and *Vibrio sp.* on ice cube samples.

Keywords: Ice Cubes, *Escherichia coli*, *Salmonella sp.*, *Vibrio sp.*, contamination.

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¹Undergraduate Medical Student, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia;

²Department of Microbiology, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia;

*Corresponding author:

Ni Nengah Dwi Fatmawati;
Department of Microbiology, Faculty of Medicine,
Universitas Udayana, Denpasar, Bali, Indonesia;
nnd.fatmawati@unud.ac.id

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INTRODUCTION

Food safety is the safety standard of food or drink which states that the food or drink consumed will not cause health problems in humans.¹ Food safety is a science strongly influenced by lifestyle and socio-cultural situations, which are indicators of contamination in food and beverages. Contamination in food and drink is defined as the presence of contamination of a substance or substances that should not be found in food or drink.¹ Several contaminants may be found in food and beverages, including chemical, physical, allergen and biological contamination.^{1,2} This kind of transmission is also often referred to as a fecal-oral transmitted disease. Waterborne diseases are diseases that are a major

concern in various countries, especially in countries or regions with insufficient water supply for the needs of the population, poor quality of water distribution (water pipes), open defecation habits and lack of quality of local sewage so that can be some indications of the poor sanitation level at the certain area that affects the quality of drinking water and water that used for other daily needs, such as for drinking water, including food, eating and cooking utensils that are washed using water that has been contaminated by infectious agents (bacteria, viruses, protozoa, etc.).^{3,4} Based on the Health Department of New Delhi Municipal Council, there are several sources of spread of pathogenic infections through drinking water, such as contaminated water sources, distribution

of unhygienic drinking water and ice cubes that are made using drinking water that has been contaminated with infectious agents.⁵

Ice cubes are one of the food products often used by the community as a complementary food, especially by the people of Indonesia. This is due to the geographical conditions and the warm climate of Indonesia, so that ice cubes are one of the food products widely used by the people of Indonesia.^{6,7} As a food product that uses drinking water as its raw material, ice cubes have the potential to be a medium for the spread of waterborne disease, whether caused by contaminated drinking water, raw materials or unhygienic production processes. Unfortunately, until now, there is no regulation regarding

quality standards, standard procedures, and quality control of ice cubes as food products made from drinking water.^{6,7}

Referring to various regulations, both from Indonesian Government Regulations and Regulations from the Ministry of Health of the Republic of Indonesia, the standard of drinking water to be consumed (including being used as raw material for ice cubes) is water that has no coliform bacteria contamination at 95% of the number of water samples that are used in checking the status of water contamination for one year or there may be contamination in 3 out of 100 samples but not found in the same sample in succession. According to the update of the Regulations from the Ministry of Health of Republic of Indonesia Number 492 of 2010, the standard quality of drinking water must not contain any coliform or *E. coli* in 100 mL water sample.⁸⁻¹⁰ several bacteria included in the pathogenic bacteria that play a role in the incidence of waterborne diseases which are *E. coli*, *Salmonella sp.*, and *Vibrio sp.* are some of the bacteria that have a high level of resistance in water with increased incidence of related diseases (health significance) such as diarrhea, typhoid fever and cholera.^{4,5}

The level of sanitation is a key factor that plays a role in the incidence of coliform bacterial contamination, both in drinking water and food products are made from drinking water, such as ice cubes. Karangasem regency is one of the regencies in Bali Province, Indonesia, which still has various problems related to infectious diseases, especially infectious diseases that are spread through food and drink due to the low level of sanitation. Based on data from the health profile of Bali Province in 2017, the population sanitation level of Bali Province reached 92.36%. Even though that level was already high, the distribution level in each district is still not evenly distributed with Karangasem Regency, which occupies the lowest position related to sanitation levels, has the percentage of population sanitation level reached 77.68%.⁸

Regarding those urgencies, start from the level of sanitation, the absence of regulations governing the quality standard of ice cubes as a food product and the lack of studies that are related

to the level of bacterial contamination of ice cubes in Karangasem Regency, researchers were interested in conducting study and research regarding the level of contamination *E. coli*, *Salmonella sp.* and *Vibrio sp.* on ice cubes in Karangasem Regency, Bali Province in 2021. This study aimed to determine the contamination of the *Escherichia coli*, *Salmonella sp.*, and *Vibrio sp.* on ice cubes at food stalls in Karangasem Regency in 2021.

METHODS

Study Design and Samples Collection

This study was conducted using cross sectional research design with simple purposive sampling as a sampling technique. Forty-two ice cube samples were taken from 8 sub-districts in Karangasem Regency, with 5-6 samples for each sub-district. The sample's inclusion criteria were; (1) Ice cubes samples were sold at food stalls in Karangasem Regency, and (2) Ice cubes have not been used at all for any consumption purposes. Meanwhile, the exclusion criteria used were; (1) The respondent from food stalls did not agree to participate in this study. Samples were taken under the aseptic condition from January 2021 to September 2021. Samples were transported properly under proper temperature to Microbiology Laboratory, Faculty of Medicine, Universitas Udayana for the identification procedures. Species identification procedures were done at Province Health Laboratory for suspected positive samples for *Salmonella sp.* and *Vibrio sp.* contamination.

Most Probable Number (MPN) Test and Bacterial Identification Procedures

The MPN test procedures were carried out according to the previous method. Lactose broth (LB) media was used in this test. The media, including the samples, were then incubated for 48 hours, the turbidity and the presence of air bubbles were observed. The MPN test results were then continued by *E. coli* identification using Eosin Methylene Blue Agar (EMBA) as previously described. Media were then incubated for 24 hours and the bacterial colonies were then observed. The samples were tested for *Salmonella sp.* and *Vibrio sp.* contaminations using *salmonella shigella* agar (SSA) and thiosulfate citrate bile

sucrose (TCBS). As previously described, the media were incubated for 24 hours and then observed for the presence of a bacterial colony.¹¹

Data Analysis

Data analysis was performed using Microsoft Excel version 2010 for Windows. Descriptive analysis was used to obtain the number of samples contaminated with *E. coli*, *Salmonella sp.* and *Vibrio sp.* through specific media Eosin Methylene Blue Agar (EMBA), *Salmonella Shigella* Agar (SSA), and Thiosulfate Citrate Bile Sucrose (TCBS). Research data obtained from each sample will be presented in the form of descriptive statistics through narratives and tables.

RESULTS

This study was conducted from January-September 2021 by taking samples of ice cubes at food stalls in 8 (eight) sub-districts in Karangasem Regency. There were 42 ice cube samples taken, and *E. coli*, *Salmonella sp.*, and *Vibrio sp.* contamination was made. This study classified bacterial contamination into contamination, and no contamination was found in each bacterium in every ice cube sample taken. In addition, the samples obtained were tested for the MPN test and received the MPN coliform index results in the ice cube samples, which can be seen in [Table 1](#). Based on this study, we found all of the samples (42 samples; 100.0%) were positive on the MPN test by the presence of air bubbles in the Durham tube and the changes of media turbidity ([Table 1](#)).

After obtaining data regarding the MPN coliform index in each ice cube sample, the identification test for the presence of *E. coli* using Eosin Methylene Blue Agar (EMBA) media was performed. The MPN test results were then cultured on EMBA media with the striking method and incubated for 24 hours in an incubator (37°C) then observed on the culture media. The results obtained were found as many as 5 samples (11.9%) were positive for *E. coli* with metallic green colored colonies, as can be seen in [Table 2](#).

To test the presence of *Salmonella sp.* and *Vibrio sp.*, ice cube samples were cultured using SSA and TCBS. Samples of ice cubes were taken as much as 10

Table 1. MPN Test Results on Ice Cube Samples.

Contamination Status	n (%)
Positive, n (%)	42 (100.0)
Negative, n (%)	0 (0.0)

Table 2. Results of Identification of *E. coli*, *Salmonella sp.*, and *Vibrio sp.* on Ice Cube Samples.

Presence of Pathogenic Organism	<i>Escherichia coli</i> (n=42)	<i>Salmonella sp.</i> (n=42)	<i>Vibrio sp.</i> (n=42)
Positive, n (%)	5 (11.9)	0 (0.0)	0 (0.0)
Negative, n (%)	37 (88.1)	42 (100.0)	42 (100.0)

µL using a micropipette and dripped on SSA and TCBS media. Then, the striking method was carried out and incubated for 24 hours in an incubator at 37°C. The results obtained were not found any contamination of *Salmonella sp.* and *Vibrio sp.* in all samples tested with no black color in the AAS media. However, on TCBS media, 3 samples were found that had yellow colonies. Still, after species identification, it was found that the findings were not a species of *Vibrio sp.*, but a species of bacteria *Listeria ivanovii* (Table 2).

DISCUSSION

E. coli is one of the coliform bacteria that can cause health problems in humans. However, *E. coli* is a normal flora in the human body, especially the large intestine; some species cause health problems, such as diarrhea, urinary tract infections, and others. *E. coli* can cause health problems depending on the location of the infection. Infections from *E. coli* can be divided into extra-intestinal and intra-intestinal infections.^{10,11} In the event of an extra-intestinal infection, such as in the urinary tract, *E. coli* can cause urinary tract infections (UTIs) which are very common in women. Meanwhile, if it occurs in the intestine, *E. coli* can cause diarrhea, depending on the infecting species. As a coliform bacterium, *E. coli* is commonly found in feces.^{12,13} But not infrequently, these bacteria are found in food and beverages due to contamination caused by various factors, ranging from washing processes to food and beverage processing. Based on the results of this study, it was found that 5 samples (11.9%) positive samples contained *E. coli*. In contrast, the other two bacteria identified in this

study, *Salmonella sp.* and *Vibrio sp.*, were not found in all samples through SSA and TCBS media identification. The results of this study are quite low when compared to other studies. A study by Nur J and Winarsih DA identified the presence of *E. coli* in ice cubes in Jakarta found 80% of the total samples identified (10 samples).¹² Almost similar results were found in the study of Cesaria O et al., which examined *E. coli* contamination in ice cubes sold in the school environment and found *E. coli* contamination in 60% of the tested samples (3 out of 5 positive samples).¹³ In addition, research by Fajriaty NR shows a difference in contamination number between ice cubes made from treated water and non-treated water.¹⁴ In the new ice using treated water, it was found that 35% of the 12 samples analyzed were positive for *E. coli*, and 65% of 27 ice cube samples made from non-treated water were confirmed to be contaminated with *E. coli*.¹⁴ This finding indicates that ice cubes that do not use treated water (e.g., well water and others) have a higher probability of *E. coli* contamination. However, in the study of Hampikyan H et al., which examined the contamination of coliform bacteria and *E. coli* in ice cubes, 7 samples (6.7%) were found positive for *E. coli*.¹⁵

In this study, the MPN test was conducted to determine the presence of coliform bacteria in ice cube samples using Lactose Broth (LB) media. Positive results in each tube were determined qualitatively by observing the presence of air bubbles in the Durham tube at the bottom of the test tube and the turbidity of the tube after adding the sample and incubating for 48 hours. Based on this study, there were no samples of ice cubes that were negative for bacterial coliform

contamination with the smallest MPN index of 12 CFU and the highest was >2400 CFU per 100 ml sample. Based on Regulations from the Ministry of Health of the Republic of Indonesia Number 492 of 2010, the standard quality of drinking water must not contain any coliform or *E. coli* in 100 mL water sample; therefore, we classified the result into positive and negative contamination.⁹ Based on this study, there were found that 42 samples (100%) were not met the water media quality standards-based Regulations from the Ministry of Health of the Republic of Indonesia Number 492 of 2010.⁹ This indicates that all of the samples taken in this study are not suitable for consumption or use for hygiene and sanitation purposes or consumption.

Based on the results of this study, there was no contamination of *Salmonella sp.* on all samples of ice cubes. The results of this study are in accordance with Putra HP which identified coconut water beverage where no *Salmonella sp.* contamination was found on the sample taken.¹⁶ However, in Febrianti PE study, which identified *Salmonella sp.* in sugarcane juice drinks, it was found that 2 out of 5 samples tested in duplicate were positive for *Salmonella sp.* contamination.¹⁷ In addition, the research conducted by Rahmawati S et al., also found similar results to Febrianti PE which found that *Salmonella sp.* in 1 of 5 drinking water samples taken at the canteen at the integrated campus of the Universitas Islam Indonesia.¹⁸ As one of the bacteria used as an indicator of the feasibility of drinking water consumption, *Salmonella sp.* It is known not to cause clinical symptoms in the first bacteremia phase so that infected patients will not take action or treatment because they are not aware of the infection. Besides being able to cause problems in the digestive tract, *Salmonella sp.* can also infect other organs such as the liver and bone marrow to cause more severe symptoms and complications.¹⁹⁻²¹

Based on the results of this study, there was also no contamination of *Vibrio sp.* on all ice cube samples used in the study. Considering *Vibrio sp.* is a bacterium often found in food samples such as fish, the author is quite difficult to find sources that discuss the findings of *Vibrio sp.* on ice cubes and drink samples. *Vibrio*

sp. is a bacterium generally found in saltwater and seafood products (seafood) because of its nature, which likes a moist environment with a sufficient level of saltiness. The presence of this bacterium in its natural environment is known to be directly proportional to the level of warmth of an environment because this bacterium likes a warm environment. However, this bacterial contamination can still occur in drinking water due to cross-contamination of materials containing *Vibrio sp.* such as seafood products (salted fish, etc.).²²

This study still has various limitations, such as the absence of a source of contamination data and the source of water used that need to be reviewed for evaluation as a basis for consideration for further research. In this study, samples that have been attempted to be carried out aseptically by burning the tools used in making ice cubes, disinfecting using a hand sanitizer, storing samples using a cool box to prevent contamination from outside and keeping the sample temperature low, and storing samples that are stored in the refrigerator at a time will be identified in the laboratory.

CONCLUSION

There were contaminations of coliform bacteria in the tested ice cube samples. In addition, *E. coli* was found in the sample, but in contrast, there was no *Salmonella sp.* and *Vibrio sp.* on ice cube samples taken from food stalls in Karangasem Regency. Further study with a more comprehensive design is needed, including the source of contaminations, further identification of coliform bacteria species and risk factors regarding lifestyle and sanitation.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding this study.

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Udayana, Denpasar, Bali, Indonesia, with the number of approval: 392/UN14.2.2.VII.14/LT/2021.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, literature search, data acquisition, data analysis, manuscript preparation until reporting the study results through publication.

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