



Factors Relating to the Presence of Escherichia Coli Bacteria in Beverages at the Area of Junior High School 1 Sukoharjo

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Abstract

Introduction: There are many traders who sell beverage at the school at a cheap price, but delicious with a variety of flavors. A preliminary study at the Junior High School 1 in Sukoharjo showed that 5 kind of beverages sold at school contained *Escherichia coli* (*E.coli*) and some problems on food handlers occurred, such as did not wear head coverings and aprons. This study aimed to determine the association between food handler hygiene, equipment sanitation, water quality and sanitation facilities with the presence of *E.coli* bacteria in beverages sold at the Junior High School 1 Sukoharjo area.

Methods: This study used a cross-sectional design. The sampling method was total sampling. The sample size was 32 traders. Beverage samples of red and white syrup milk ice, macapuno coconut ice, milk tea ice, grass jelly ice, iced tea, milkshakes, hot tea, lemon tea ice, young coconut ice, sugar cane ice, Thai tea, chocolate ice, sour sugar ice. Data were collected through observation, interviews and laboratory tests using total germ numbers. Chi Square and Fisher Exact tests were used for the analysis.

Results: There were associations between food handler hygiene, equipment sanitation, water quality, and sanitation facilities with the presence of *E.coli* bacteria in beverage in the Junior High School 1 Sukoharjo area, with the p values of 0.001, 0.001, 0.004 and 0.015 respectively.

Conclusion: For reducing *E. coli* infection among the students, food handler hygiene, equipment sanitation, water quality and sanitation facilities at the school should be improved., especially at Junior High School 1 Sukoharjo.

Keywords: Beverages, Escherichia Coli Bacteria, Food Handler, Hygiene, Sanitation.

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Introduction

The widespread sale of various beverages at low prices and delicious tastes has made beverages as the favorite drink among the public, from adults to children. We can easily find these drink sellers, from the roadside to the school area. The ease of licensing at schools and no complicated licensing process has made many traders decide to sell in school areas. Various types of beverages that are

sold in school areas include red syrup milk ice, white syrup milk ice, *macapuno* coconut ice, milk tea, grass jelly ice, iced tea, milkshakes, creamer, warm tea, lemon tea ice, young coconut ice, ice sugar cane, Thai tea, chocolate ice and also sour sugar ice.

Apart from the cheap prices and good taste, the other things to consider are the food handler hygiene and sanitation of the equipment used. Personal hygiene is the

cleanliness and health of an individual to prevent the emergence of disease in oneself and others, both physically and psychologically.¹

According to Sundari (2013), sanitation of eating and drinking utensils is an effort to maintain health and prevent disease originating from eating and drinking utensils. Supervision of food sanitation needs to be carried out starting at before food production, during the processing, storage, and transportation, until the food and drinks ready for consumption. Good water quality is also a consideration when making beverages because the main raw material for selling beverages is water.

Water is the main need for living creatures to fulfill all daily needs such as drinking, cooking and washing. The water used must meet health requirements.¹ Regulation of the Minister of Health of the Republic of Indonesia Number 492/Menkes/Per/IV/2010 concerning Drinking Water Quality Requirements states that drinking water is declared safe for health if it meets the physical, microbiological, chemical and radioactive requirements in the mandatory and additional parameters. Drinking water is the water used for human consumption. The requirements for drinking water are tasteless, odorless, colorless, does not contain harmful microorganisms, and does not contain heavy metals.²

Water consumed as drinking water must meet health requirements, where the water does not contain *E.coli* bacteria.² The presence of *E.coli* bacteria indicates that the water has been contaminated with microbes.³ *E.coli* is normal flora in the human intestine and will cause disease if it enters other organs or tissues. Certain types of *E.coli* can cause diarrheal illness.⁴

In 2019, World Health Organization (WHO) stated that diarrhea is the main cause of death in children and kills around 525,000 children every year. Diarrhea in children is caused by bacterial infections, malnutrition, unclean water sources and lack of knowledge about preventing diarrheal illness. Based on recapitulation data from the Sukoharjo District Health Service for 2019 - 2021, there was an increase in diarrheal illness cases from 2019 - 2020. In 2019

there were 12 cases and in 2020 there was 19 cases. In 2021 the number of cases was the same, 19 cases of diarrheal illness. Diarrhea cannot be separated from the hygiene and sanitation of food equipment.

E.coli is a bacteria that is often used as the main standard for food hygiene, because this bacteria is an early indication of other bacterial contamination that can cause disease, especially diarrheal illness. *E.coli* can not only contaminate snacks and drinks, but also pollute water sources, making contaminated water unfit for consumption.⁵

Snack food and drinks are widely served. This distribution of snack vendors is often found at schools, from elementary to the high schools. The number of traders tend to increase due to the large number of enthusiasts. Similar things are found in all schools, the presence of street vendors selling snacks and drinks. The food and drinks are sold at affordable cheap prices.⁶

Drinks is quite risky for the hygiene and bacterial content. Marhamah and Huda (2014) found that ice syrup sold at state elementary schools in Bandar Lampung was of poor quality because it did not meet the requirements of the Indonesian Ministry of Health. No. 492/MENKES/PER/IV/2010.⁷ Siti Maimunah (2020) study showed that 1 out of 4 samples of beverage found on the Muslim Captain's Street contained *E.coli* bacteria ranging from 2,800 cells/100 ml so they were included in the criteria of being unfavorable. In this category, these beverage were not suitable for consumption and did not meet the criteria set out in the Indonesian Ministry of Health No.492/Menkes/Per/IV/2010 (Coliform and Escherichia coli/100 ml) .⁸

Middle school children have high levels of activities while at school, especially outdoor activities during the break. This high level of activities often causes thirst, and they buy drinks that are sold at school. At the Junior High School 1 Sukoharjo area, drinks are popular among the students as they are relatively cheap, easy to reach, and easy to accept on the tongue. The drinks also have various flavors. In general, junior high school students consume snacks or drinks without paying

attention to the cleanliness and ingredients contained in them.

Based on a preliminary study at Junior High School 1 Sukoharjo, the most favorite drinks were iced tea, iced lemon tea, iced chocolate, iced sugar and sour sugar, iced sugar cane, iced young coconut, and iced Thai tea. From the 7 drinks of samples taken, 5 drinks were positively contained *E.coli*. The five drinks are iced tea, iced lemon tea, iced chocolate, iced young coconut and iced Thai tea, while iced sugar cane and iced sugar are negative for *E.coli*. According to the Regulation of the Minister of Health of the Republic of Indonesia Number 492/Menkes/Per/IV/2010 concerning Drinking Water Quality Requirements, in the microbiological parameters, the maximum level allowed of *E.coli* bacteria per 100 ml sample is 0.⁹

Contamination of bacterial contaminants is likely caused by the hygiene of food traders who do not pay attention to the cleanliness aspects. The initial preliminary survey found that of the 7 food traders at Junior High School 1 Sukoharjo, 4 food traders did not wear head coverings, 5 did not wear aprons, 4 did not wear masks, and 7 did not wear gloves when handling the food. In the aspect of equipment sanitation, it was found that 7 food traders did not wash equipment using running water, 5 food traders did not dry equipment after washing. In terms of water quality, 7 food traders used refillable water or gallon water. In terms of sanitation facilities, of the 7 food traders, 6 persons used plastic or plastic bins as trash cans and 1 person used a basket trash can without a lid. Based on the description above, researchers are interested in conducting research on factors related to the presence of *E.coli* bacteria in snacks in the Junior High School 1 Sukoharjo area.

The aim of this research was to determine the association between food handler hygiene, equipment sanitation, water quality and sanitation facilities with the presence of *E.coli* bacteria in beverages sold at the Junior High School 1 Sukoharjo area. There was not much research has been done on the presence of *E.coli* bacteria in beverages sold at

junior high schools.

Method

This study was done in a cross sectional design, which measure the food handler hygiene, equipment sanitation, water quality and sanitation facilities in relation to the presence of *E.coli* bacteria in beverages at the Junior High School 1 Sukoharjo area. These variables are studied at the same time to determine the association between these variables.

The food handler hygiene variable was measured using a questionnaire and an observation sheet of 28 question items, with a total score of 28. The food handler hygiene categories were meet (score ≥ 14) and not meet the requirements (score < 14). The equipment sanitation variable was measured using a questionnaire and observation sheet 16 question items, with a total score of 16. The equipment sanitation category was eligible (score ≥ 8) and not eligible (score < 8). Water quality variables are measured using a questionnaire with good and bad categories. The sanitation facility variable was measured using a questionnaire with good and bad categories. The variable for the presence of *E.coli* bacteria was measured through a laboratory test, with categories that met the requirements (negative for *E.coli*) and did not meet the requirements (positive for *E. coli*).

The sample in this study used total sampling technique. There are 32 beverage traders in the Junior High School 1 Sukoharjo area, all 32 traders were sampled.

Data collection techniques were carried out using observations and interviews regarding food handler hygiene, equipment sanitation, water quality and sanitation facilities, while the presence of *E.coli* bacteria was carried out through bacteriological quality testing of beverage in collaboration with the Sukoharjo Regional Health Lab. The tools used in laboratory tests are sterile petri dish, sterile measuring pipettes 10 ml and 1 ml, Erlenmeyer, Incubator, Autoclave, Scales, Test tubes, Test tube rack, Beaker glass, Spatula, Measuring cup, Colony counter, Water bath, Thermometer, Bottle sterile and stationery.

The materials used were 32 drinks, 70% alcohol, 0.9% NaCl, spirit lamps and matches, cotton, aluminum foil, brown paper, hemp rope, masks and medical gloves, labels, plastic bags, and aquadest. Standard Operational Procedures were divided into 2 stages. The first one was the sampling stage which includes buying drinks in the Junior High School 1 Sukoharjo area, gave a label to each drink package that had been purchased, put the drink sample into a cool box then took it to the laboratory and checked the sample for the total number of germs.

The second stage was the inspection of the total plate number on the drink samples, which included: sterilized the table and tools for handling the samples, prepared the drink samples that had been purchased. The examiners wore a mask and medical gloves soaked in 70% alcohol, wrapped the test tube, Erlenmeyer flask, measuring cup, petri dish, and pipette with paper and covered it with aluminum foil then put them in the oven at 160°C for 2 hours, prepared a spirit lamp, and sterilized test tubes for the number of samples to be tested, then arranged them on the tube rack. Each tube was sequentially marked as 10-1, 10-2, 10-3, 10-4, 10-5 and K as the dilution code. Then prepared a sterilized petri dish for the number of samples tested.

On the back of the petri dish a mark was given according to point 6. Another petri dish was marked with a control. A diluent solution was prepared in the form of Peptone Water in an Erlenmeyer, then filled all the test tubes with 9 ml of distilled water. The sample was aseptically pipetted 1 ml into a sterile bottle which contains 9 ml of sterile 0.9% NaCl and then homogenized by pipetting in and out 25 times, so that a sample of 10-1 is obtained. Pipet 1 ml from retail 10-1 then put into the first tube containing 9 ml of sterile 0.9% NaCl and homogenized by pipetting in and

out 25 times and the first tube is called dilution 10-2. Pipetted 1 ml of the first tube and put into the second tube and homogenized by pipetting in and out 25 times and the second tube is called a 10-3 dilution, The same treatment was carried out on the second to fifth tubes. In the fifth tube, 1 ml was pipetted and put into a glass beaker (not used).

The sixth tube was not filled with samples because as a control, 1 ml of each diluent was pipetted and put into all the petri dishes that have been coded. Each petri dish was filled with 15-20 ml of Nutrient Agar media with a temperature of 45-55°C. Each petri dish was rotated clockwise with the aim of homogenizing the sample. After solidifying, the cup was incubated at 35-37°C for 2 x 24 hours in an inverted position and the number of growing colonies was observed and counted.

The observation sheets and questionnaires were used for data collection. The validation and reliability of the questionnaires had been tested on beverage traders in front of PT Sritex Sukoharjo. Beverage traders at PT Sritex Sukoharjo had the same characteristics (age, highest education and gender) as beverage traders at Junior High School 1 Sukoharjo. Validity and reliability tests were carried out by distributing questionnaires and observation sheets to 40 beverage sellers around PT SRITEX SUKOHARJO. Data analysis was done by chi square tests with 95% Confidence Intervals.

Results

Food Handler Hygiene

Table 1 shows that 12 food handlers (37.5%) did not qualify for the hygiene requirements. There were 20 (62.5%) food handlers qualify for the hygiene requirement.

Table 1. Food Handler Hygiene

No	Handler Hygiene	Amount	Percentage (%)
1	Not Qualify	12	37.5
2	Qualify	20	62.5
Total Number		32	100

Equipment Sanitation

Table 2 shows that there were 14 respondents (43.8%) not qualify for the requirements for sanitizing equipment and 18 respondents (56.2%) who qualify for the equipment sanitation.

Table 2. Equipment Sanitation

No	Equipment Sanitation	Amount	Percentage (%)
1	Not Qualify	14	43.8
2	Qualify	18	56.2
Total Number		32	100

Water Quality

Table 3 shows that there are 11 (34.4%) of good water quality and 21 (65.6%) of not good water quality.

Table 3. Water Quality

No	Water Quality	Amount	Percentage (%)
1	Good	11	34.4
2	Not Good	21	65.6
Total Number		32	100

Sanitation Facility

Table 4 shows that among the samples, 5 (15.6%) had good sanitation facilities and 27 (84.4%) had not good sanitation facilities.

E.coli Bacterial Content Variables

Table 5 shows that the bacterial content of the beverages were positive on 17 samples (53.1%) and negative on 15 (46.9%) samples.

Table 4. Sanitation Facility

No	Sanitation Facility	Amount	Percentage (%)
1	Good	5	15.6
2	Not Good	27	84.4
Total number		32	100

Table 5. Escherichia Coli Bacterial content of the beverages

No	Escherichia Coli Bacterial content	Amount	Percentage (%)
1	Positive	17	53.1
2	Negative	15	46.9
Total Number		Not Qualify	100

Association between Food Handler Hygiene and E.coli Bacteria Content in Beverages

Table 6 shows that among the non-qualified food handlers, 91.7% had positive *E. Coli* bacteria on their beverages that they sold, while among the qualified food handlers, 70% of the beverages had negative *E. Coli* content in their beverages that they sold. The results of the chi square test obtained a p value of $0.001 < \alpha (0.05)$, which mean that there was an association

between food handler hygiene and the *E.coli* bacteria content in the beverage sold at Sukoharjo State Middle School area.

Association between Equipment Sanitation and E.coli Bacteria Content in Beverage

Table 7 shows that among the non-qualified equipment sanitation, 85.7% had positive *E. Coli* bacteria on their beverages that they sold, while among the qualified

Table 6. Association between Food Handler Hygiene and Bacterial Content in Beverages

Food Handler Hygiene	Escherichia Coli Bacteria Content				Total		p
	Positive		Negative		N	%	
	N	%	N	%			
Not Qualify	11	91.7	1	8.3	12	100	0.001
Qualify	6	30.0	14	70	20	100	

equipment sanitation, 72.2% of the beverages had negative E. Coli content in their beverages that they sold. The results of the chi square test obtained a p value of $0.001 < \alpha (0.05)$, which mean that there

was an association between equipment sanitation and the *E.coli* bacteria content in the beverage sold at Sukoharjo State Middle School area.

Table 7. Association Between Equipment sanitation and Escherichia Coli Bacterial Content in Beverage

Equipment sanitation	Escherichia Coli Bacteria				Total		p
	Positive		Negative		N	%	
	N	%	N	%			
Not Qualify	12	85.7	2	14.3	14	100	0.001
Qualify	5	27.8	13	72.2	18	100	

Association between Water Quality and E.coli Bacteria Content in Beverage

Table 8 shows that among the good quality of water users, only 18.2% had positive *E. Coli* bacteria on their beverages that they sold, while among the users of bad water quality, 71.4% of the beverages had positive E. Coli content in their beverages that they sold. The results of the chi square test obtained a p value of $0.004 < \alpha (0.05)$, which mean that there was an association between the use of water quality and the *E.coli* bacteria content in the beverage sold at Sukoharjo State Middle School area.

Association between sanitation facility and E.coli Bacteria Content in Beverage

Table 9 shows that among the good sanitation facility, all of the beverage had negative *E. Coli* bacteria, while on the bad sanitation facility, 63 % had positive *E. Coli* bacteria on their beverages that they sold. Because the chi square test requirements were not met, Fisher's exact test was used and the result show a p-value of 0.015, which was $< \alpha (0.05)$. Thus, there was an association between sanitation facilities and *E.coli* bacteria content in beverage sold in the Sukoharjo State Middle School Area.

Table 8 Association Between Water Quality and *E.coli* Bacteria Content in Beverage

Water Quality	Escherichia Coli Bacteria				Total		p
	Positive		Negative		N	%	
	N	%	N	%			
Good	9	81.8	2	18.2	11	100	0.004
Bad	6	28.6	15	71.4	21	100	

Table 9 Association Between sanitation facility and *E.coli* Bacteria Content in Beverage

Sanitation facility	Escherichia Coli Bacteria				Total		P
	Positive		Negative		N	%	
	N	%	N	%			
Good	0	0	5	100	5	100	0.015
Bad	10	37.0	17	63.0	27	100	

Discussion

Food Handler Hygiene

From the research results it is known that there were 12 food handlers (37.5%) who were not qualified for the hygiene requirements. There were 20 (62.5%) food handlers qualify for the hygiene requirements. A food handler is a worker who handles food starting from preparation, processing, storing, transporting and serving food. Food handler hygiene is the food handler's efforts to maintain clean hands, work clothes, clean hair and personal health.¹⁰ From the research results, it was found that there were 5 food handlers who did not wear headgear, 10 people did not wear aprons and 5 people smoked.

Apart from that, it was also found that food handlers had not implemented handler hygiene, such as washing their hands using running water and washing their hands with soap. Dirty or contaminated hands can transfer pathogenic bacteria and viruses in the body, feces or other sources to food. Hand washing is the main thing that must be done by workers involved in handling food. Washing hands with soap followed by rinsing will remove many microbes found on the hands.¹¹

Equipment sanitation

From the research results it is known that there were 14 respondents (43.8%) did not qualify the requirements for sanitizing equipment and 18 respondents (56.2%) who qualified for equipment sanitation. Equipment sanitation can be seen based on the equipment washing process and the use of equipment that is designed for single use.¹⁰ Food equipment plays an important role in food quality. If clean food is stored in equipment that does not meet the requirements, it can result in contamination of the food.¹² From the aspect of equipment sanitation, it was found that 7 traders did not wash equipment using running water and 5 traders did not dry equipment after washing.

Water Quality

From the research results, it is known

that there were 11 (34.4%) food sellers who used good water quality and 21 (65.6%) used bad water quality. Water quality plays a significant role in contaminating food or eating utensils. Water contains more than 90 contaminants which can have a bad effect on health, one of which is diarrhea or digestive disorders. In daily activities the water used for cleaning will decrease in terms of the number of germs produced.¹³

Most people do not know about good hygiene practices, especially the impact of the lack of clean water used for washing cutlery. If the water used to wash cutlery is contaminated by germs, it can result in contamination of the cutlery by bacteria which can later result in contamination of the food served.¹⁴

Sanitation Facility

From the research results, it is known that there were 5 (15.6%) sellers who had good sanitation facilities and 27 (84.4%) had bad sanitation facilities. Based on laboratory examination, it was found that 17 samples examined were contaminated with *E.coli*, which was not in accordance with the Decree of the Minister of Health, Number: 1098/Menkes/SK/VII/2003, about the amount of *E.coli* content in food equipment samples must be 0/ 100 ml per sample.

Research conducted by Nur Mayang, et al (2017) at Candimulyo Jombang, resulted in identifying the number of *E.coli* bacteria in iced tea drinks using the MPN method with 5 series of tubes resulted in all drinks being unfit for consumption with a percentage of 100%.⁴

E.coli Bacterial Content

From the research results, it is known that the bacterial content that does not meet the requirements is 17 (53.1%) and the bacterial content that meets the requirements is 15 (46.9%). Based on laboratory examination, it was found that 17 samples examined were contaminated with *E.coli*, which is not in accordance with the Decree of the Minister of Health, Number: 1098/Menkes/SK/VII/2003, which states that the amount of *E. coli* content in food equipment samples must be 0/ 100 ml per sample.

Research conducted by Nur Mayang, et al (2017) resulted in identifying the number of *E.coli* bacteria in iced tea drinks sold in Candimulyo Jombang Hamlet using The MPN method with 5 series of tubes resulted in all drinks being unfit for consumption with a percentage of 100%.⁴

Association between Food Handler Hygiene and E.coli Bacteria Content in Beverage

There was an association between food handler hygiene and the *E.coli* bacteria content in beverage sold in the Sukoharjo State Middle School area. The vendor hygiene affected the bacteriological quality, the lower the vendor's hygiene, the higher the chance that beverage contain bacteria or exceed the safe limit for microbial contamination.

Most of the food sellers at the SMP N 1 Sukoharjo area were females. Female traders had already worn head coverings because they wore the hijab and already wear aprons. Male traders did not wear aprons and smoked when handling drinks, had long and dirty nails. Some sellers and food handlers who did not wash their hands using running water, only used water in a bucket and used it for many times. However, contamination of drinks could occur from dirty hands and clothes that were worn from the place of residence to the place of sale, so it did not rule out the possibility that the clothes used can be a source of contamination for the drinks being sold. Traders' hygiene did not meet the requirements because traders did not know about the importance of maintaining the cleanliness of food and drinks

This research was also in line with research by Fitka Romanda, et al (2016), which showed that there was an association between personal hygiene of food handlers and the presence of *E.coli* in food in the food processing place (TPM) buffer area of Adi Soemarmo Airport, Surakarta ($p = 0.000$; $p < 0.05$), with the level of closeness of the association between the two being ($C = 0.4777$; $C < 0.5$).¹⁶

Association between Equipment sanitation and E.coli Bacteria Content in Beverage

The chi square test resulted in a p-value of $0.001 < \alpha (0.05)$, which mean that

there was an association between equipment sanitation and the content of *E.coli* bacteria in beverage sold in the Sukoharjo State Middle School area.

Traders still use equipment that did not meet the requirements. Equipment that did not meet these requirements was caused by the method of drying for equipment which used a rag and is used repeatedly which can also transfer bacteria from dirty equipment to clean equipment. Apart from that, the use of water to wash equipment that was not flowing mean that only using a bucket and the water was not changed, sometimes the color of the water in the bucket was cloudy because the selling location was far from a clean water source, which can transmit germs to the equipment that would be washed the next. Where equipment was stored openly and did not turn over could also increase the risk of contamination by dust or other dirt. Attention are needed on always changing equipment dryers regularly, using running water and soap and also placing the equipment upside down to avoid contamination with dust or other bacteria.

In line with the research conducted by Herwin, et al (2023) which stated that the variable sanitation of equipment has a 1.858 times greater risk for the presence of *E.coli* bacteria.¹⁷ One of the causes of bacterial contamination of drinking water was equipment sanitation and maintenance of processing equipment.¹⁸

Association between Water Quality and E.coli Bacteria Content in Beverage

The chi square test results showed a p-value of $0.004 < \alpha (0.05)$, which mean that there was an association between water quality and the content of *E.coli* bacteria in beverage sold in the Sukoharjo State Middle School Area.

Based on the observations of the water quality of all traders, the water was odorless and colorless/turbid. Good water conditions include providing water for washing hands separately from washing equipment, such as using running water from a tap and a separate bucket for washing hands and washing equipment. This type of water was considered as good if it used boiled water, so it was free from bacteria.¹⁹ Clean water quality that does not meet the requirements indicates that

the water is dirty and contaminated with microorganisms. If water/buckets that have been contaminated with *E. coli* bacteria are used to wash equipment, it is likely that the equipment will be contaminated with these bacteria.¹¹

Association between Sanitation Facilities and The Content Of E.coli Bacteria In Beverage

Because the chi square test requirements were not met, Fisher's exact test was used and the p-value of 0.015 was obtained. This meant that there was an association between sanitation facilities and the *E.coli* bacteria content in beverage sold in the Sukoharjo State Middle School Area.

The results of this research were in line with research by Afriyanti (2019), which showed that there was an association between the condition of sanitation facilities and the presence of *E.coli* bacteria in drinks sold in elementary school canteens in Semarang City with a p value of 0.034. Providing sanitation facilities is very important to support the production of snacks for sale and keep the environment safe.¹⁹

Conclusion

There were associations between food handler hygiene, equipment sanitation, water quality, and sanitation facilities with the presence of *E.coli* in beverages sold in Junior High School 1 Sukoharjo. Training is needed for the student to choose healthy food and drinks.

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