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Original Article

Identification of Pathogenic Bacteria in Tofu Water and Sugarcane Water in Pontianak City

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ABSTRACT

Tofu water and sugar cane juice are trendy drinks, especially in Pontianak City. Pathogenic bacterial contamination in tofu water and sugar cane juice can be a severe threat if the immune system is weak. This research aims to identify pathogenic bacteria in Pontianak City tofu water and sugar cane water. The methods used are culture methods and biochemical tests. The research samples were tofu water and sugar cane juice sold in the community. The sample was selected using a purposive sampling technique. In this study, the bacteria found in the identification of bacteria in tofu water and sugar cane water were Klebsiella pneumoniae, Shigella dysenteriae, Staphylococcus aureus, Enterobacter aerogenes, Salmonella typhi, and Escherichia coli. Bacteria dominate, while in sugarcane juice, it is dominated by Escherichia coli, followed by Klebsiella pneumoniae. The research results can also be the basis for better policies and preventive measures related to the safety of the quality of tofu water and sugar cane juice in Pontianak City. Therefore, this research can be relevant to environmental protection and public health in Pontianak.

Keywords: *Tofu Water, Sugarcane Water, Pathogenic Bacteria*

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INTRODUCTION

Water is the main need for the lives of living creatures, whether humans, animals, or plants[1], so water quality directly impacts the environment and public health. Decreased water quality can become a place for microorganisms to live and carriers of microorganisms that are dangerous to human health. Therefore, the presence of pathogenic microorganisms in the water must be monitored. Generally, these microorganisms are pathogenic bacteria that can cause digestive tract infections[3].

Pathogenic bacteria can cause disease, especially digestive tract infections [4,5]. Some

pathogenic bacteria that can cause disease include Vibrio cholerae, which causes cholera [6]; Shigella dysenteriae, which causes shigellosis or bacillary dysentery in humans with a high prevalence rate[7]; and Salmonella typhi, which causes typhoid fever, a systemic infection with symptoms such as fever for a long time [8].

Tofu water and sugar cane juice are very popular drinks, especially in Pontianak City. Tofu water comes from yellow soybeans, which are yellowish-white, similar to milk. Tofu water drinks have high nutrition, especially the protein content in them[9]. Sugarcane juice comes from the juice of sugar cane stalks, making it have a naturally sweet taste and is liked by many people. Sugarcane juice is sold in various forms, namely pressed sugarcane directly or in packaged form. These two drinks are often found in restaurants or food stalls and even on the side of the road, so they have a relatively high risk of exposure to pathogenic bacteria. These bacteria can spread by contaminating ingredients used in making tofu water and sugar cane juice drinks. For example, ice cubes are used using raw water, which causes contamination of tofu water and sugar cane juice drinks.

Information regarding the potential for pathogenic bacterial contamination in tofu water and sugar cane water in Pontianak City is still limited. Identifying pathogenic bacteria is important to determine the potential risks of consuming contaminated tofu water and sugar cane juice[10]

The research results will educate the public about the presence of pathogenic bacteria in tofu water and sugar cane juice. They can prevent the dangers of pathogenic bacteria for public health. The research results can also be the basis for better policies and preventive measures related to the safety of the quality of tofu water and sugar cane juice in Pontianak City. Therefore, this research can be relevant to environmental protection and public health in Pontianak.

METHOD

This research is descriptive with a cross-sectional design, carried out in April-May 2023. The identification method used was laboratory culture at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Health Polytechnic, Ministry of Health, Pontianak. The research samples were tofu water and sugar cane juice, which were sold by the community. Samples were selected using a purposive sampling technique with the criteria that street vendors sold tofu juice and sugar cane juice on the side of the road and did not move from place to place and sold from 10.00 to 14.00 in the Pontianak City and Southeast Pontianak sub-districts so that 43 tofu water samples and 70 water samples were obtained. Sugarcane.

The culture method's bacterial identification stage begins with inoculating the sample into enrichment media. From the growth enrichment medium, Gram11 staining was carried out. Gram-positives were inoculated on BAP and MSA media. Meanwhile, Gram-negatives were inoculated on MC, SSA, and EMB media. Then, from each specific characteristic obtained, it is continued to biochemical media. The tools used include test tubes, Erlenmeyer flasks, Petri dishes, spirit lamps, circular lines, needle tubes, glass objects, ovens, autoclaves, and microscopes. The materials used are lactose broth (LB), tryptic soy broth (TSB), selenite broth (SB), MacConkey's agar (MCA), blood agar plate (BAP), mannitol salt agar (MSA), Salmonella Shigella agar (SSA) media.), sulfide indole motility (SIM), methyl red (MR), Voges-Proskauer (VP), Simmon's citrate (SC), and sugars (glucose, lactose, maltose, mannitol, and sucrose).

RESULTS

This study was conducted in April – May 2023. The identification method used was culture, and continued with biochemical tests. Biochemical testing is a test to identify bacteria and determine the metabolic activity of microorganisms [12]. The metabolic properties of bacteria in biochemical tests are observed from the interactions produced by metabolites with chemical reagents and their ability to use certain compounds as carbon sources and energy sources. Several biochemical tests that can be used are the IMViC, SIM, TSIA, and confectionery fermentation tests [13].

Table 1. Growth characteristics of Gram-negative bacteria from samples

Add-On Media	Coloring Grams	Selective Media	Biochemical Media	Bacteria
LB: (+)	Shaped stem , colored red , Gram- negative	MC: colored red young , mucoid EMB: colored green-black , sized medium-large	TSIA: acid/acid, gas (+), H_2S (-); urease: (+); driving license (- - +); MR: (-); VP: (+); SC: (+); glucose : (+); lactose : (+); maltose : (+); mannitol : (+); sucrose : (+)	*Klebsiella pneumonia*
LB: (+)	Shaped stem , colored red , Gram- negative	MC: Flat , dark -pink , dry , non-mucoid EMB: Green metallic-dark	TSIA: Acid/Acid, gas (+); H_2S (-); urease: (+); Driver's License: (- + +); MR: (+); VP: (-); SC: (-); glucose : (+); lactose : (+); maltose : (+); mannitol : (+); sucrose : (+)	*Escherichia coli*
LB: (+)	Shaped stem , colored red , Gram- negative	MC: Flat , dark -pink , dry , non-mucoid EMB: Green metallic-dark	TSIA: Acid/Acid, gas (+); H_2S (-); urease: (+); Driver's License: (- + +); MR: (+); VP: (-); SC: (-); glucose : (+); lactose : (+); maltose : (+); mannitol : (+); sucrose : (+);	*Enterobacter aerogenes*
S B: (+)	Shaped stem , colored red , Gram- negative	SSA: Clear, middle black	TSIA: Alkaline /Acid, gas (+); H_2S (+); urease: (-); Driver's license: (+ - +); MR: (+); VP: (-); SC: (-); glucose : (+); lactose : (-); maltose : (+); mannitol : (+); sucrose : (-);	*Salmonella typhi*
S B: (+)	Shaped stem , colored red , Gram- negative	SSA: Clear, small, round MC: Small, smooth, clear	TSIA: Alkaline /Acid, gas (-); H_2S (-); urease: (-); Driver's License: (- + +); MR: (+); VP: (-); SC: (-); glucose : (+); lactose : (-); mannitol : (-); sucrose : (-);	*Shigella dysenteriae*

Table 1 shows that tofu water and sugar cane juice samples were positively infected with bacteria such as Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, and Shigella dysenteriae. Klebsiella pneumonia is a Gram-negative bacterium, rod-shaped, red in color, pink in MC media, mucoid, and tends to stick together. The results of the indole-MR-VP-SC (IMViC) test on Klebsiella pneumoniae bacteria showed results (-) (-) (+) (+). Escherichia coli is a Gram-negative bacterium, rod-shaped, red in color, flat on MC medium, dark pink, dry non-mucoid, indole-MR-VP-SC (IMViC) test results (+) (+) (-) (-). Enterobacter aerogenes is a Gram-negative bacterium, rod-shaped red; on MC media, it looks the same as Klebsiella pneumoniae bacteria; the difference between the two bacteria is seen on EMB media on Klebsiella pneumoniae with dark pink and mucoid colonies, on biochemical media Enterobacter aerogenes able to hydrolyze urea while Klebsiella pneumoniae bacteria are not able to hydrolyze urea. Salmonella typhi is a Gram-negative bacterium, rod-shaped, and red; on SSA media, it looks clear, the middle is black, and the results of the indole-MR-VP-SC (IMViC) test are (-) (+) (-) (-). Shigella dysenteriae is a Gram-negative bacterium, rod-shaped red; on SSA media, it looks clear, small, and round; the results of the indole-MR-VP-SC (IMViC) test are (+) (+) (-) (-).

Table 2. Growth characteristics of Gram-positive bacteria from samples

Add-On Media	Grams	Selective Media	Catalase	Coagulase	Bacteria
TSB : (+)	Form round ; Color purple ; Gram s (+)	MSA: Yellow, yellow zone Dossier Beta hemolysis	Catalase (+)	Coagulase (+)	*Staphylococcus aureus*

Based on Table 2, from the 43 samples of tofu water, nine samples were positive for Staphylococcus aureus, and the sugar cane juice was negative. The catalase test differentiates Staphylococcus from Streptococcus, while the coagulase test differentiates pathogenic Staphylococcus (Staphylococcus aureus) and non-pathogenic Staphylococcus.

Table 3. Results of identification of pathogenic bacteria in tofu water and sugar cane water

Bacteria	Water knows		Sugarcane juice	
	Positive	Negative	Positive	Negative
Klebsiella pneumoniae	15 of 43 samples (34.88)	28 of 43 samples (65.12)	14 of 70 samples (20%)	56 of 70 samples (80%)
Escherichia coli	0 out of 43 samples (0%)	0 out of 43 samples (100%)	15 of 70 samples (21.42)	55 of 70 samples (78.58)
Enterobacter aerogenes	2 of 43 samples (4.65%)	41 of 43 samples (95.35)	8 of 70 samples (11.42)	62 of 70 samples (88.58)
Salmonella typhi	8 of 43 samples (18.60)	35 of 43 samples (81.4%)	4 of 70 samples (5.71%)	64 of 70 samples (94.29)
Shigella dysenteriae	0 of 43 samples (0%)	0 out of 43 samples (100%)	6 of 70 samples (8.5%)	64 of 70 samples (91.5%)
Staphylococcus aureus	9 of 43 samples (21%)	34 of 43 samples (79%)	0 out of 70 samples (0%)	0 out of 70 samples (100%)

Based on Table 3, the results showed that samples of tofu water and sugar cane water were contaminated with pathogenic bacteria, including Klebsiella pneumoniae, Escherichia coli, Enterobacter aerogenes, Salmonella typhi, Shigella dysenteriae, and Staphylococcus aureus, which could potentially threaten public health. The results of the identification of tofu water were that Klebsiella pneumoniae bacteria were found in 15 samples out of 43 samples (34.88%), while in sugar cane juice samples, it was found in 14 samples out of 70 samples (80%). The next identification result was that no Shigella dysenteriae bacteria were found in tofu water, whereas in sugar cane water, six samples out of 70 samples (8.5%) were found. The identification results in tofu water found Staphylococcus aureus bacteria in 9 out of 43 samples (21%), while in sugar cane juice samples, no bacteria was found in 0 out of 70 samples (0%). In the tofu water samples, Enterobacter aerogenes bacteria were found in 2 out of 43 samples (21%), while in sugar cane water, bacteria were found in 8 out of 70 samples (11.42%). Other identification results in tofu water showed that Salmonella typhi bacteria were found in 8 out of 43 samples (18.60%), while in sugar cane juice, four bacteria in 70 samples (5.71%) were found. In tofu water samples, 0 out of 43 samples (0%) of Escherichia coli bacteria were not found, while in sugarcane juice samples, 15 of 70 samples (21.42%) contained E. coli bacteria.

DISCUSSION

Identification using the culture method was carried out to see the contamination of pathogenic bacteria in tofu water and sugar cane juice in Pontianak City. Six types of disease-causing pathogenic bacteria were found, namely Klebsiella pneumonia, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Shigella dysenteriae, and Staphylococcus aureus, which can cause different diseases. Pathogenic bacterial contamination in tofu water and sugar cane juice is a waterborne disease. Waterborne disease is a disease caused by consuming water contaminated by pathogenic microorganisms [14].

Klebsiella pneumoniae is a pathogen often found to cause various infections, including pneumonia, urinary tract infections, and bacteremia[15]. The increasing number of K. pneumoniae cases showing resistance to multiple types of drugs was recognized by the World Health Organization in 2017 as a serious threat to public health [16].

Shigella is a Gram-negative bacterium whose cells are rod-shaped and do not move. Shigella dysenteriae spreads from infected individuals via the fecal-oral route, including drinking water, which can be contaminated by various pollution factors such as sufferer feces, food, or through intermediaries such as flies. After entering the human body, Shigella dysenteriae attacks by invading mucosal epithelial cells. These bacteria then exit the phagocytic cell vacuole, reproduce or multiply, spread into the cytoplasm, and finally spread to other adjacent cells [18].

Staphylococcus aureus is a cocci-shaped bacterium with a diameter of 1 µm, which, on staining, is Gram-positive; when viewed under a microscope, it looks like a grape[19]. Staphylococcus aureus can cause infectious diseases in hair follicles and sweat glands, boils, and wound infections. This bacterium has low invasiveness, being involved in many skin infections [19].

Over the last three decades, Enterobacter aerogenes has been identified as an opportunistic type of pathogenic bacteria that is resistant to many antibiotics and has become a serious problem in the hospital environment [12]. One of the diseases caused by Enterobacter aerogenes is the most common UTI.20

Salmonella typhi is a pathogenic bacteria that causes typhoid fever, a systemic

infectious disease characterized by prolonged fever, bacteremia, and inflammation, which can damage the intestines and liver[2]. Typhoid fever is an infectious disease that spreads worldwide and is still a major health problem in developing and tropical countries such as Southeast Asia, Africa, and Latin America [21]

Escherichia coli is an opportunistic microorganism generally found as normal flora in the human colon. The characteristics of E. coli are unique: it can cause primary infection in the intestine and potentially cause infection in various body tissues outside the intestine[22]. Infections in the intestine can cause diseases such as enteritis, gastroenteritis, hemorrhagic colitis, bacillary dysentery, and enteric fever, and the main symptom is diarrhea. Infections outside the intestines are also often found, such as cystitis, urinary tract infections, respiratory tract infections, sepsis, and meningitis[23].

The discovery of pathogenic bacterial contamination in tofu water and sugar cane juice sold in Pontianak City could pose a serious threat to the community. People in Pontianak often consume tofu water and sugar cane juice, which results in the risk of disease arising from drinking tofu water and sugar cane juice, which is contaminated with pathogenic bacteria.

CONCLUSION

Identification of bacteria in tofu water and sugarcane water found contamination with Klebsiella pneumoniae, Shigella dysenteriae, Staphylococcus aureus, Enterobacter aerogenes, Salmonella typhi, and Escherichia coli, where in tofu water, Klebsiella pneumoniae dominated, while in sugarcane water it was dominated by Escherichia coli followed by Klebsiella pneumoniae. It is hoped that the research results will make people aware of how to maintain personal hygiene to prevent transmission and will become the basis for policies to implement better sanitation regarding the safety of the quality of tofu water and sugar cane juice drinks sold in the Pontianak City area.

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