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COLIFORM CONTAMINATION LEVEL OF WASHING WATER AT STREET VENDORS TENT STALLS IN YOGYAKARTA

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ABSTRACT

Food quality in Indonesia is significantly influenced by street vendors, particularly from a microbiological aspect. The use of unclean washing water in these processes can cause the contamination of tableware, increasing the risk of foodborne diseases. Therefore, this research aimed to determine the level of coliform contamination in the washing water used by street vendors. A total of 21 samples were collected from different locations, isolated, and identified morphologically and biochemically. These samples were resuscitated in 1% peptone medium for 12 hours and inoculated on Chromocult Coliform Agar (C.C.A.) medium. Purified coliform colonies were identified morphologically and biochemically to the genus level and confirmed using API 20E K.I.T. reagents. The results showed coliform contamination levels ranging from 5.1 x 10^5 to 2.7 x 10^8 CFU/mL, exceeding the quality standard. Confirmation results from API 20E assay kit found bacteria contaminants with ID ≥ 95%, including Escherichia coli and Klebsiella pneumoniae subsp. pneumoniae, and Pantoea spp. Pneumoniae, and Pantoea spp. These findings underscore the pressing need to address the risks associated with tableware contamination at street vendor stalls

INTRODUCTION

Street vendors, as informal traders, play a vital role in the regional and city economic system. They operate small-scale businesses, selling goods and food in public places such as sidewalks (Permatasari, 2021; Pranata & Purbadi, 2020). In Yogyakarta,

approximately 10,000 street vendors are spread across six sub-districts. These vendors typically set up stalls in tents in commercial areas with public facilities, including tourist attractions, campuses, and hospitals (Winoto, 2017). The limited availability of equipment and washing water sources can facilitate contamination and increase the risk of germ transmission and contamination (Lado*et al*., 2020).

The availability of washing water is essential in tent stalls, as it is a vital medium for washing cooking utensils and food. Furthermore, water quality significantly influences the cleanliness of tableware, food products, and safety (Sari, 2016). Observations show that many street vendors use water collected in buckets, often needing to change it regularly during the washing process or to rinse cutlery before washing. These practices result in unhygienic washing of water, creating an environment conducive to bacterial growth (Agustine *et al.*, 2021; Marisdayana *et al.*, 2017). Various coliform groups, such as *Escherichia coli*, *Klebsiella*, *Enterobacter aerogenes, Citrobacter freundii, Salmonella*, and Serratia, are commonly found in contaminated water (Sunarti, 2015). Coliforms can be grouped into faecal and non-faecal coliforms, distinguished by their ability to ferment lactose at different temperatures (44.5°C and 35°C, respectively) (Naratama & Santoso, 2017). In the environment, coliforms are the critical indicator of microbial contamination in waters and the possible presence of other pathogens. According to Indonesia's Minister of Health Regulation Number 32 of 2017 on Environmental Health Quality Standards and Water Health Requirements for Sanitary Hygiene Purposes on biological parameters, the maximum allowable total coliform level is 50 CFU/mL, while *E. coli* should not be present at any detectable level (0 CFU/mL).

Biological contamination by microbes causes foodborne disease due to improper food safety practices (Wardani, 2019). This can lead to health problems experienced by consumers, such as diarrhoea and food poisoning due to pathogenic bacteria (Sunarya, 2019; Mulya, 2021). Diarrhoea is a disease with high morbidity and mortality rates that occurs in various countries, including Indonesia, with 80-90% of cases caused by pathogenic microorganisms (Handayani, 2018; Mafazah, 2013). The Ministry of Health of Indonesia stated that 44.4% of diarrhoea cases occurred in 2020 (Kemenkes, 2021). According to data from the WHO, as many as 2.2 million people were affected by diarrhoea. The CDC estimates that there are 128,000 people with diarrhoea, with 3,000 people dying due to food contamination. Evaluation of the sanitation program needs to

be done to determine the effectiveness of the cleanliness of production equipment used by street vendors through microbiological methods as a commonly used analytical method in the food industry (Agustine, 2021). Therefore, the objective of the research was to determine the level of coliform contamination in the washing water used by street vendors in Yogyakarta, highlighting the urgent need for improved sanitation practices.

MATERIALS AND METHODS

Sample collection

Samples of tableware washing were collected from seven tent stalls of street vendors in Yogyakarta, from three types of washing buckets (Figure 1), and stored in sterile falcon tubes. Subsequently, these samples were then taken to the esteemed Industrial Biotechnology Laboratory of Universitas Kristen Duta Wacana, renowned for its expertise, for isolation and identification based on their morphological and biochemical characteristics.

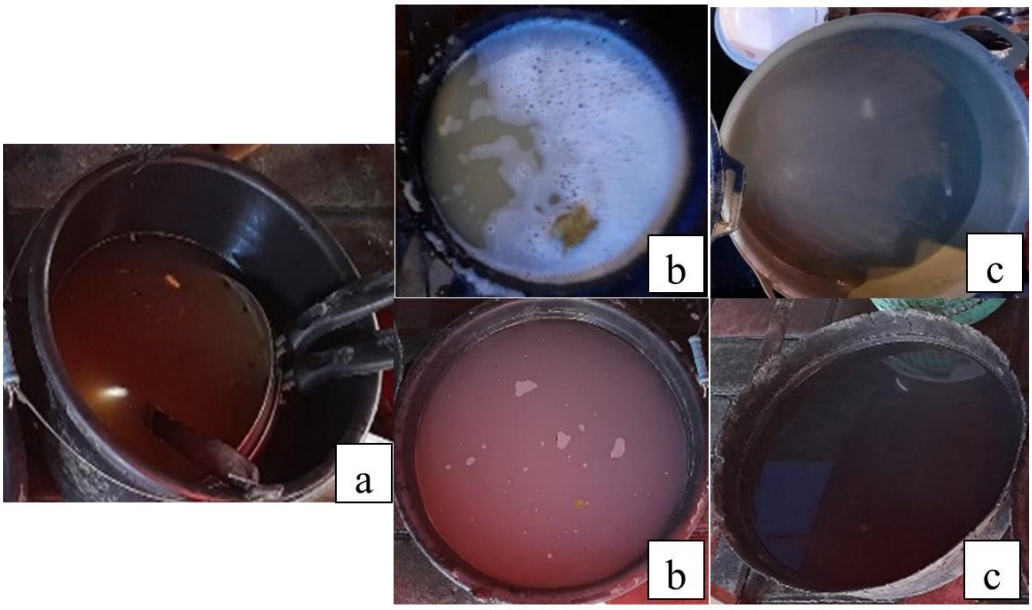

Figure 1. Three types of washing buckets at Street Vendors' Tent Stalls (a). Soaking Bucket, (b). Saponification bucket, and (c). Flushing bucket.

Resuscitation and isolation of coliform

A 10 ml sample of washing water was centrifuged for 15 min at 6000 rpm. The precipitate was collected, placed in 10 ml of 1% peptone water, and incubated for 6-12 hours. Subsequently, 1 ml of the previous culture was diluted up to 10^{-6} using 9 ml of

0.1% peptone water (Verawati *et al.*, 2019). A total of 0.1 ml from dilutions 10^{-4}, 10^{-5}, and 10^{-6} was inoculated onto Chromocult Coliform Agar (CCA) medium and incubated at 37°C for 24 hours. The coliform colonies, characterized by red colour, were meticulously counted based on the standard range of 30-300 CFU (Tahya *et al*., 2018), ensuring the accuracy of the results. Coliform isolates from each sample were then purified, transferred, and collected on the Brain Heart Infusion Agar (BHIA) medium for further assays (Yunus *et al.*, 2017).

Identification of morphological and biochemical characteristics

Morphological tests were conducted using Gram staining to identify the cells' shape, colour, and size. The preparations were sequentially treated with reagents A (crystal violet), B (iodine), C (acetone), and D (safranin) according to the Gram staining procedure (Cappucino, 2016), followed by observation under a microscope at 1000x magnification. Coliform groups were identified by their red colour, indicating Gram-negative bacteria. Colonies with uniform rod-shaped cells were subjected to a comprehensive range of biochemical tests including motility, Methyl Red–Voges Proskauer (MR-VP), indole, citrate, urease, and Triple Sugar Iron Agar (TSIA) (Budiarso *et al*., 2021). The thoroughness of these tests ensured the accuracy of the results. Biochemical test results with the highest percentage conformity, based on Brenner and Farmer's identification key (2015), were further confirmed using the API 20E assay kit.

Coliform identification using the API 20E assay kit

The confirmation stage was conducted using the API 20E system (Biomereoux) to identify isolates at the species level within the *Enterobacteriaceae* family. The Analytical Profile Index (API) identification test compared the biochemical reactions of the tested microorganisms with a database of known characteristics. Isolates were collected using an inoculation loop (ose) and suspended in 5 ml of physiological saline solution (NaCl 0.85%). The meticulous process of inoculating the pure cultures in the physiological saline suspension into 20 test wells using a drop pipette was crucial for ensuring the accuracy of the results. The wells for decarboxylation of the amino acid arginine by arginine dihydrolase (ADH), decarboxylations of the amino acid lysine by lysine decarboxylase (L.D.C.), decarboxylations of the amino acid ornithine by ornithine

decarboxylase (O.D.C.), production of hydrogen sulfide (H2S), test for the enzyme urease (U.R.E.) tests were filled with the culture suspension, while the wells for utilization of citrate as only carbon source (C.I.T.), Voges-Proskauer (V.P.), and test for the production of the enzyme gelatinase which liquefies gelatin (G.E.L.) tests were filled with culture suspension and sealed with mineral oil to facilitate anaerobic reactions. The plates were incubated for 24-48 hours at 37°C. After incubation, T.D.A. reagent was added to T.D.A. wells, VP1 and VP2 reagents were incorporated into V.P. test wells, and James reagent was added to IND test wells. The results were then interpreted using A.P.I.W.E.B. software to determine species name and I.D. percentage. These results provided a similarity profile index based on the biochemical characteristics of the tested isolates (Budiarso *et al*., 2021).

RESULTS AND DISCUSSION

Isolation of coliform in washing water

Coliform was detected in washing water samples of street vendors using a CCA medium. This differential selective medium contained Salmon-GAL and X-Glucuronide (a chromogenic mixture), sorbitol, peptone, sodium chloride, sodium pyruvate, disodium hydrogen phosphate, sodium dihydrogen phosphate, and ribitol, which inhibits the growth of Gram-positive bacteria (Naratama & Santoso, 2020). The total coliform colonies, as shown in Table 1, averaged more than 105 CFU/mL after the resuscitation stage. The lowest count was 5.1 x 105 CFU/mL, while the highest total colonies reached 2.7 x 10^8 CFU/mL. These results were higher than those Budiati (2015) reported, where values ranged between 3.2 x 10^4 CFU /mL and 9 x 10^5 CFU /mL. The isolation and selection results for suspected coliform colonies in all samples exceeded the established quality standards, indicating the need for further research and immediate action.

Table 1. Coliform Colonies from Washing Water Samples of Street Vendors

Sample	Bucket	Total Suspected Coliform Colonies CFU/mL
S1	E1	1.0 x 10^7
	E2	1.5 x 10^7
	E3	2.7 x 10^8
S2	E1	7.1 x 10^6
	E2	1.3 x 10^6
	E3	9.0 x 10^6
S3	E1	4.9 x 10^6

Sample	Bucket	Total Suspected Coliform Colonies CFU/mL
	E2	3.5×10^6
	E3	6.3×10^6
	E1	7.8×10^6
S4	E2	7.5×10^6
	E3	2.3×10^7
	E1	3.4×10^7
S5	E2	2.1×10^7
	E3	2.8×10^7
	E1	1.9×10^7
S6	E2	7.8×10^6
	E3	6.3×10^6
	E1	7.5×10^6
S7	E2	3.0×10^6
	E3	5.1×10^5

Description: S1-S7: sample origin; E1: washing bucket; E2: first rinsing bucket; E3: second rinsing bucket.

The data showed that the average number of suspected coliform in the first washing and rinsing bucket was lower compared to the second bucket. This difference is likely due to the residual soap content in the first bucket, which can kill bacteria. Surfactants in dish soap can inhibit cell wall synthesis, disrupt metabolic processes, and ultimately cause cell lysis (Setiawati, 2019). The presence of suspected coliform indicated contamination due to poor hygiene practices, posing a risk of bacterial spread on tableware and food (Teramura et al., 2017). Importantly, further morphological and biochemical identification tests are needed to determine the identity of these contaminant bacteria, highlighting the importance of continued research in this area.

Morphological and biochemical identification of coliform

The morphological identification results indicated that the isolates had a bacillus shape and were classified as Gram-negative bacteria. A total of 33 isolates were selected for further biochemical testing. The biochemical identification test, based on the identification key table from Bergey's Manual of Systematics of Archaea and Bacteria (Brenner & Farmer, 2015), revealed the bacteria to be Escherichia coli and Klebsiella sp, as shown in Figure 1. The motility test revealed that Klebsiella sp. was non-motile due to the absence of flagella, while Escherichia coli was motile, possessing flagella for movement. The results of the I.M.V.I.C., urea, and T.S.I.A. tests for the suspected colonies were consistent with the identification key from Brenner & Farmer (2015). These findings are significant as they contribute to our understanding of bacterial identification and could potentially impact future research in the field.

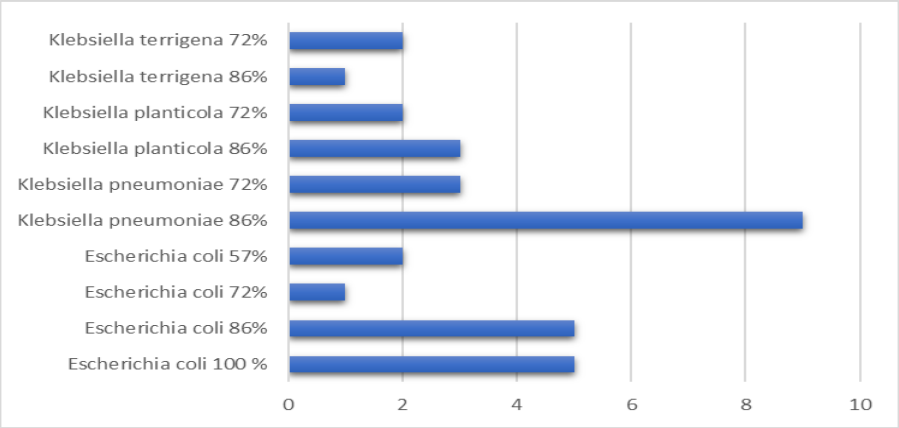

Figure 2. Coliform Identification Results Based on Percentage Conformity with

According to the identification key from Brenner & Farmer (2015), out of 33 isolates identified biochemically, five isolates were selected for confirmation testing based on the highest percentage of agreement. These isolates represented *Escherichia coli, Klebsiella pneumoniae, Klebsiella planticola,* and *Klebsiella terrigena* by API 20E assay kit (Table 2, Figure 2). In the test comparing two bacterial identification systems, API 20E and Bis NEG-D, the concordance probability values varied between 95% and 100% for API 20E and between 79.3% and 100% for Bis NEG-D. The API 20E system is better than the Bis NEG-D system (Rakotovao-Ravahatra et al., 2020). The API 20E system has an 80% success rate for identifying gram-negative bacilli (Kusumaningsih et al., 2021). The API 20E kit has proven to be highly reliable, providing a sense of security in its use, and can replace automated systems when used according to the manufacturer's instructions and good laboratory practices. It can also identify members of the Enterobacteriaceae family down to the species and subspecies level (Maina *et al.*, 2014). These results confirm that API 20E provides identification based on an extensive database and is a miniature test system that is standardized, fast, safe, and easy to use.

The identification of *Escherichia coli, Klebsiella pneumoniae* subsp. pneumoniae, and *Pantoea* sp. in the water samples were attributed to improper tableware washing techniques (Marisdayana *et al.*, 2017), including not regularly changing the water in the bucket after each wash (Budiati, 2015). Field observations revealed that street vendors often washed 15 to 20 dirty dishes without replacing the water, and some lacked standard procedures for water replacement, allowing dirty tableware to be stacked or soaked for extended periods (Rahayu *et al.*, 2018). *These findings underscore the urgent need for improved hygiene practices in our communities. Escherichia coli* contamination in the

water environment was measured at 1.7 x 10^3 CFU/mL (Oktavianto, 2014) and on tableware, it ranged from 11-70 colonies/cm^2 (Suryanti, 2019).

Table 2. Coliform Colonies Identification Results from Washing Water Samples Using the API 20E Assay Kit.

Isolate Code	Confirmed API 20E Test	% ID
S2E3MP2	*Pantoea* spp	98.6%
S3E2MP1	*Klebsiella pneumoniae* ssp. pneumoniae	94.8%
S3E2MP2	*Klebsiella pneumoniae* ssp. pneumoniae	94.5%
S4E1UG2	*Escherichia coli*	99.5%
S7E2MP1	*Klebsiella pneumoniae* ssp. pneumoniae	97.3%

VERY GOOD IDENTIFICATION

Strip	API 20 E V5.0
Profile	5 1 4 4 5 7 2
Note	

Significant taxa	% ID	T	Tests against			
Escherichia coli 1	99.5	0.96				

Next taxon	% ID	T	Tests against			
Kluyvera spp	0.4	0.53	LDC 25%	SOR 25%	AMY 99%	

Figure 3. Biochemical Test Confirmation Results of *Escherichia coli* using the API 20E Assay Kit.

Escherichia coli, *Klebsiella pneumoniae*, and *Pantoea* sp. found in washing water can be pathogenic. *Escherichia coli* causes diarrheal diseases. Depending on their virulence factors, virulent *E. coli* strains cause non-inflammatory diarrhea (watery diarrhea) or inflammatory diarrhea (dysentery with stools that usually contain blood, mucus, and leukocytes) (Evans and Evans, 1996). *Klebsiella pneumoniae* causes severe pneumonia. *Klebsiella* is a multidrug-resistant species and causes infections in the lungs, urinary tract, bloodstream, wounds or surgical sites, and brain (Chang et al., 2021). *Pantoea agglomerans* is detected in cases involving pneumonia that is not effectively treated and can be life-threatening for patients suffering from pneumonia (Kaur et al., 2020). Certain strains of *Escherichia coli* are known to cause diarrhea in humans, with 31 out of 98 infants affected due to factors such as improper washing practices, lack ofof clean water, and poor handler hygiene. The primary factors contributing to bacterial contamination in 21 water samples included irregular water changes during washing activites and the absence of a designated rinsing bucket (Musawir & Arsin, 2014). The Enterobacteriaceae family, which includes these bacteria is known to cause infections and

frequently contaminates street food or open environment. Their presence is an indicator of poor hygiene practices, particularly in hawker food settings. The risk of bacterial contamination increases when cooking utensils are washed without running water or when unboiled water is used (Parawidnyaningsih *et al*., 2023). This highlights the need to improve sanitation by regularly replacing washing water to minimize cross-contamination through tableware, which could potentially lead to health problems.

CONCLUSION

In conclusion, this research detected coliform contamination in all washing water samples from street vendors, underscoring the need for immediate action. The total contamination levels ranged from 5.1 x 10^5 CFU/mL to 2.7 x 10^8 CFU/mL, with the bacteria identified as *Klebsiella pneumoniae, Escherichia coli,* and *Pantoea* sp. These contamination levels exceeded the environmental health quality standards and water health requirements for sanitary hygiene, emphasizing the importance of addressing this issue promptly.

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