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Detection of Coliforms and Enteric Pathogens in Favorite Snack Food Sold in Yogyakarta City

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ABSTRACT
Favorite snack food is very popular to Yogyakarta's residents, such as cilok, skewered meatballs, and dumplings. The processing and serving processes of these food does not pay attention to hygiene aspects, therefore, it is necessary to monitor the presence or absence of coliform bacteria and enteric pathogens that often cause digestive disorders. A total of 30 samples were collected from each food from different locations. These samples were then enumerated on a CCA medium to grow all types of coliforms and enteric pathogens. The resulted colonies were then selected on SSA, SMAC, and DFI Agar medium to obtain a single isolate, which were biochemically tested until their genus levels were identified using API 20E. Based on the identification results of 30 food samples, the contamination levels obtained were as follows, Escherichia coli (16.6%), Klebsiella pneumoniae (13.3%), Yersinia enterocolitica (13.3%), Pantoea spp (6.6%).), Aeromonas hydrophila (6.6%), Enterobacter cloacae (6.6%), Serratia marcescens (6.6%), Bordetella / Alcaligenes / Moraxella spp (6.6%), Serratia liquefaciens (3.3%), Proteus mirabilis (3.3%), Shigella spp (3.3%), and Ewingella Americana (3.3%). Based on these findings, it is necessary to be cautious of street food in Yogyakarta City.

Keywords: *Favorite , Snack Food, Coliform, Enteric Pathogens.*

1. INTRODUCTION

Snacks or ready-to-eat food according to the Food and Agriculture Organization (FAO) is known to be processed by vendors/traders and consumed directly without further processing. Generally, these foods are traded on the most visited streets or public places by street vendors (PKL) [1]. This trade solves social and economic problems, especially in developing countries, since it absorbs labour in densely populated rural and urban areas. This food is sold at a relatively cheap price , making it affordable for many people. Generally, this trade is informal and requires no business license, regulation, and protection for consumers. As a result, food vendors pay less attention to hygiene factors, starting from material preparation, the processing, and serving, which can cause health problems in the community [2]. Disease cases due to consumption of food contaminated by pathogenic microorganisms (foodborne pathogens) have become a serious concern in the world and occur in many developing countries, including *Acinetobacter spp., Campylobacter jejuni, Citrobacter koseri, C. freundii, Enterobacter sakazakii, E. cloacae, Escherichia coli* O157: H7, *Klebsiella oxytoca, K. pneumoniae, Listeria monocytogenes, Salmonella Enteritidis, Salmonella Typhimurium, Shigella sonnei, Vibrio cholerae,* and *Yersinia pestis*. Therefore, foodborne diseases have become serious challenges to food security and public health in the world [3,4,5].

Yogyakarta is well known as a student city in Indonesia. This was supported by jawapos.com news source on 9 March 2018, which stated that Yogyakarta has more than 100 universities with

350,000 students. Meanwhile, data from dapo.dikdasmen.kemdikbud. pemprov. DIY stated that there were 7,938 students in the 2020/2021 academic year. Therefore, the large number of students in Yogyakarta has become the driving force for their community's economy. A krjogja.com source stated that the monthly spending amount by students was approximately 600 billion and part of their money was used for buying snacks, such as cilok (balls made from tapioca flour), skewered meatballs, and dumplings. Based on the high consumption of street food, especially by students and the lack of knowledge about food safety by vendors, studies related to foodborne diseases are very important. The purpose of this study is to determine the presence or absence of coliform bacteria and the enteric pathogens in favorite snack food in Yogyakarta, such as cilok, dumplings, and skewer meatballs.

2. MATERIALS AND METHOD

2.1. Sample Collection

Snack samples tested in this study were food consumed by Yogyakarta's residents, especially their students, such as cilok, dumplings, and skewer meatballs. Each sample was collected from 10 street vendors in different locations, and chosen based on the most visited by patronizers, namely those located around schools or campuses, and several public places. A total of 30 samples were collected in pre-sterilized containers and tested immediately in the laboratory in less than one hour of collection.

2.2. Enumeration and isolation of coliform bacteria and enteric pathogens

Snack food samples were weighed as much as 10 g and placed in 90 ml of Buffered Peptone Water (BPW) medium, then incubated for 18-24 hours at 37°C [6]. 1 ml of cell culture from BPW medium was diluted to 10^{-7} using 0.1% 9 ml peptone water. Cell cultures were homogenized using vortex, then 0.1 ml was taken from dilutions of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} to be inoculated on the Chromocult Coliforms Agar (CCA). In this medium, the coliforms and enteric bacterial colonies grew and produced different colours of colonies, such as red, dark blue, light blue, and white based on their ability to use the existing substrate in the CCA medium. The dark blue colonies form by *E. coli* were isolated using a CCA medium by streak plate method to obtain a single colony. The red colour colonies were found to be *Enterobacter spp, Citrobacter spp,* and *Klebsiella spp* [7,8], which were transferred to Sorbitol MacConkey Agar (SMAC) and E. sakazakii Agar (DFI) Agar medium using the streak plate method, then incubated for 24-48 hours at 37ºC. The SMAC Agar was used as a selective differential medium to detect the presence or absence of pathogenic bacteria namely, *Escherichia coli* O157: H7, and indicated by a colorless colony. While DFI Agar medium was used to detect the presence of Enterobacter sakazakii, indicated by green colonies [9,10]. Then, White colonies were transferred to Salmonella Shiglla Agar (SSA) medium by streak plate method to differentiate *Salmonella sp., Shigella sp., Escherichia coli,* and *Proteus sp.* microorganisms within 24-48 hours of incubation at 37ºC. The coliform and pathogenic enteric bacteria from each of the grown samples were then transferred to Brain Heart Infusion Agar (BHIA) medium to be stored as a collection of isolates for further testing [11,12].

2.3. Confirmation of coliform bacteria and enteric pathogens using API 20 E

The confirmation stage was carried out through biochemical testing using the API 20E (Biomereoux) kit to identify the species level in the Enterobactrriaceae family. The isolates were first purified using CCA medium by streak plate method, then grown on BHI Agar medium for 18-24 hours at 37ºC. Cell culture was then taken using ose and suspended in a physiological salt medium (5 ml of 0.85% NaCl) with a turbidity level equivalent to 0.5 McFarland solution. The culture solution was dropped into 20 biochemical test dish containing the API 20 E (Biomeriux) testing kit using a sterile pasteur pipette. In ADH, LDC, ODC, H2S, and URE dish which had an underlined code, the cell suspension was only dropped to reach half of each dish, and mineral oil was then added to fill the cupule. This process was carried out to create anaerobic condition. In CIT, VP, and GEL dish,

which also had an underlined code, the cell suspension was dropped into each dish until it was filled. Meanwhile, in the part with no code, the cell suspension was dropped to reach only the half of the dish. Subsequently, API 20E strips were incubated at 37°C for 18-24 hours, and used in the test for positive and negative results. The dishes in TDA test were added with 1 drop of TDA reagent, the VP dishes were added with 1 drop of VP1 and VP2 reagents, and the IND dishes were added with 1 drop of James reagent. Furthermore, an additional test was carried out on the GLU dish with the addition of Nit1 and Nit2 one drop each for NO_2 testing. When the result was negative, it showed a yellow colour and added with Zn powder for N_2-gas testing. The positive and negative results from each test were confirmed using API web software and also to identify the isolated species. The results showed the identification of the species and the magnitude of their similarity in Enterobacteriaceae family were collected by the API 20 E system, Biomereoux [13,14,15].

3. RESULTS AND DISCUSSION

3.1. Contamination of coliform bacteria and enteric pathogens in snacks.

To detect the presence of coliform bacteria and the enteric pathogens contamination in snack food sold in Yogyakarta City, various types of food were selected from different sampling locations visited by many people, especially the students. The samples were first given pre-enrichment treatment using BPW medium to perform resuscitation of injured bacterial cells. Bacteria contained in food were injured when heat treated and produced a false negative result in the test for food quality, therefore, needed to be grown first in a pre-enrichment medium to nourish the injured cells before testing for bacteria presence. The BPW was chosen as a pre-enrichment medium in testing snack food based on Cox, et. al., (2017) test result, which showed that BPW was the best medium compared to others [6]. The CCA medium was chosen to explore the presence or absence of coliform and enteric pathogens contamination in the snack food studied, due to their high suitability for biochemical characters. Coliform bacteria were members of the *Enterobacteriaceae* family which were known to ferment lactose, produce acids, and gases. Furthermore, the *Enterobacteriaceae* members were grouped according to their ability to break down the β-galactosidase and ß-glucoronidase enzyme substrates. The bacterial colonies that were known to ferment ß-galactosidase enzyme substrate producing pink to red, or mauve colour were from the genus *Citrobacter*, *Enterobacter*, and *Klebsiella*. Meanwhile, the *E. coli* produced a dark blue to violet colour, due to its ability of using both substrates [7,8,16]. The *Enterobacteriaceae* members that have pathogenic properties were not capable of using ß-galactosidase substrates, however, they used ß-glucoronidase substrates producing a white, light blue, or transparent colonies, and were namely *Salmonella*, *Shigella*, and *Yersinia* [17, 18]. The differences in the colonies that appeared on the CCA medium of the *Enterobacteriaceae* members were shown in Figure 1.

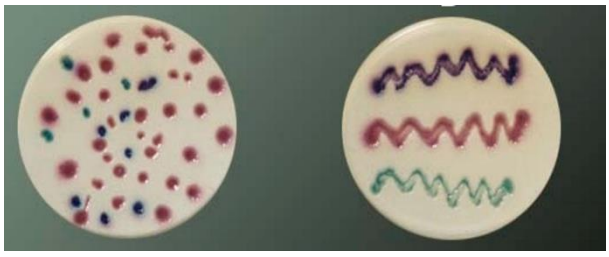

Figure 1. Appearance of coliform bacteria and Enterobacteriaceae Colonies on CCA medium. Dark blue colonies: candidates of *E. coli*, Light blue colonies : candidates of *Salmonella, Shigella, Yersinia*, Red colonies : candidates of *Citrobacter, Enterobacter, Klebsiella*

Based on the enumerated results of coliform bacteria growing on CCA medium, there were differences in the number of colonies from each sample tested. The highest coliform contamination level was obtained on the skewer meatball samples with a colony number of more than 9 log CFU/g. Meanwhile, the cilok and dumplings samples had varying levels of contamination ranging from 2-8 log CFU/gr and 2-7 log CFU/gr, respectively. The

contamination level for all tested samples were shown in Figure 2. Ferawati (2017) stated that the aerobic plate count test on skewer meatballs in Payakumbuh, West Sumatra, Indonesia showed a contamination rate almost the same as the coliform contamination rate in Yogyakarta, which was around 7-8.6 log CFU/gr. Similarly, Fauziah (2017) showed that meatballs and cilok snacks sold around the Jember University had a contamination rate of 2-10 log CFU/gr. Most of the bacterial contamination rates in the samples tested had exceeded the threshold limit for microbial contamination (1x105 CFU/gr or 5log CFU/gr) set by the National Agency of Drug and Food Control (BPOM) [19,20]. The cases of coliform bacteria contamination were also found as much as 75% on Ready to eat hot dogs sold by street vendors in Rio Grande do Sul, the Southern-most State of Brazil [21].

3.2. Identification results of coliforms and enteric pathogens

The identification of bacteria types was carried out biochemically using API 20E (Figure 3). Based on the identification results of 30 samples, various types of coliform and enteric bacteria were found to be potential pathogenic with contamination levels as shown in Figure 4. The API 20E (Biomreoux) kit was chosen for biochemical identification due of its high accuracy. The test results for suitability showed that 572 strains from the Enterobacteriaceae family had 81.6%, 440 strains of *E. coli* had 91%, and 44 pathogenic *E. coli* isolates from lettuce samples had 100% [22,23].

Based on the identification results of all samples, 12 bacteria contaminations were observed namely, *Escherichia coli, Klebsiella pneumoniae, Yersinia enterocolitica, Pantoea spp, Aeromonas hydrophila, Enterobacter cloacae, Serratia marcescens, Bordetella / Alcaligenes / Moraxella spp, Serratia liquefaciens, Proteomonas hydrophila, Enterobacter cloacae, Serratia marcescens, Bordetella / Alcaligenes / Moraxella spp, Serratia liquefaciens, Proteus mirabilella, Shorus mirabilp,* and *Ewingella Americana*. Based on the calculation results from each sample (Figure 4), the highest contamination level in all samples was dominated by *E. coli, Klebsiella pnemoniae*, and *Yersinia enterolitica*, while others had less than 10 %. All the types of bacteria found in this study were from Enterobacteriaceae family living in the digestive tract of humans and animals, they included the *Escherichia, Salmonella, Shigella, Klebsiella*, and *Serratia*. Some of them also lived as normal flora in the digestive tract causing diseases, such as *Salmonella, Shigella, Yersinia*, and some *E. coli* strains known to be the most abundant facultative anaerobic bacteria in the human digestive tract. Enterotoxigenic *E. coli* (ETEC) was one of the most common causes of diarrhea in children in developing countries, and had six intestinal pathotypes, such as Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* and, enteroinvasive *E. coli*, (including the *Shigella* strain) [24,25,26].

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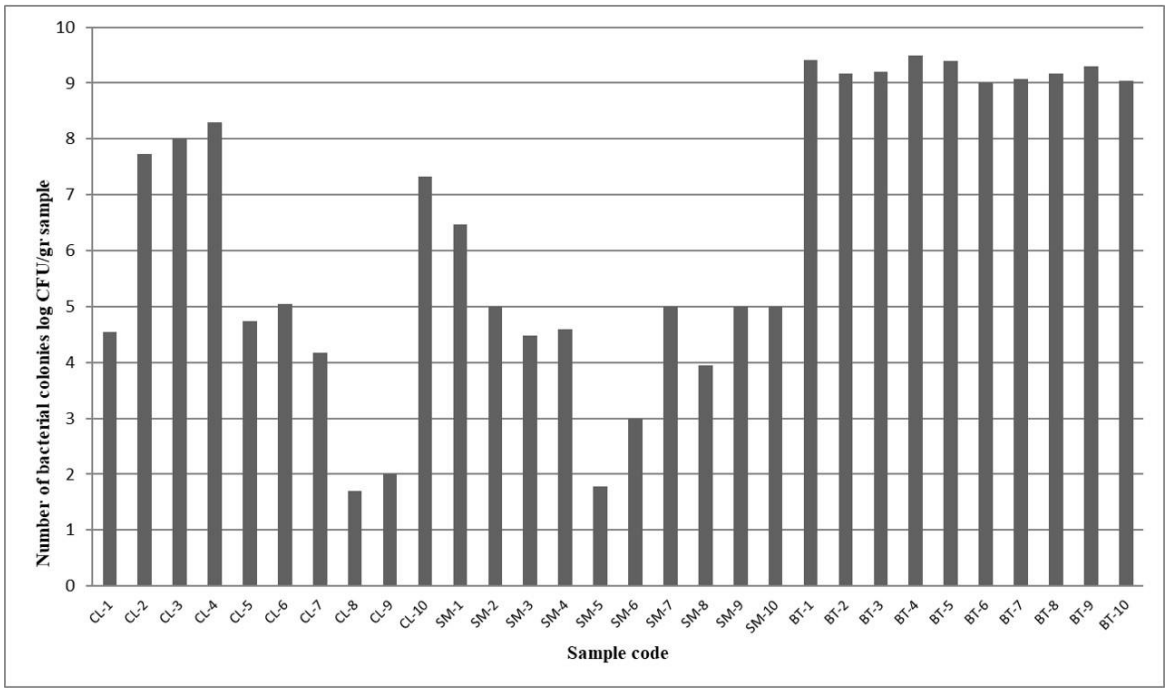

Figure 2. Enumeration results of coliform bacteria from snack food samples on CCA medium. (CL) Cilok (SM) Dumplings, (BT) Skewer Meatballs.

(a)

(b)

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

Significant taxa	% ID	T	Tests against			
Escherichia coli 1	92.6	0.54	H2S 1%			

Next taxon	% ID	T	Tests against			
Citrobacter freundii	4.0	0.31	CIT 75%	IND 1%	SAC 99%	

Figure 3. Biochemical test candidate of *Escherichia coli* using API 20E (a) and result confirmation *E. coli* (b).

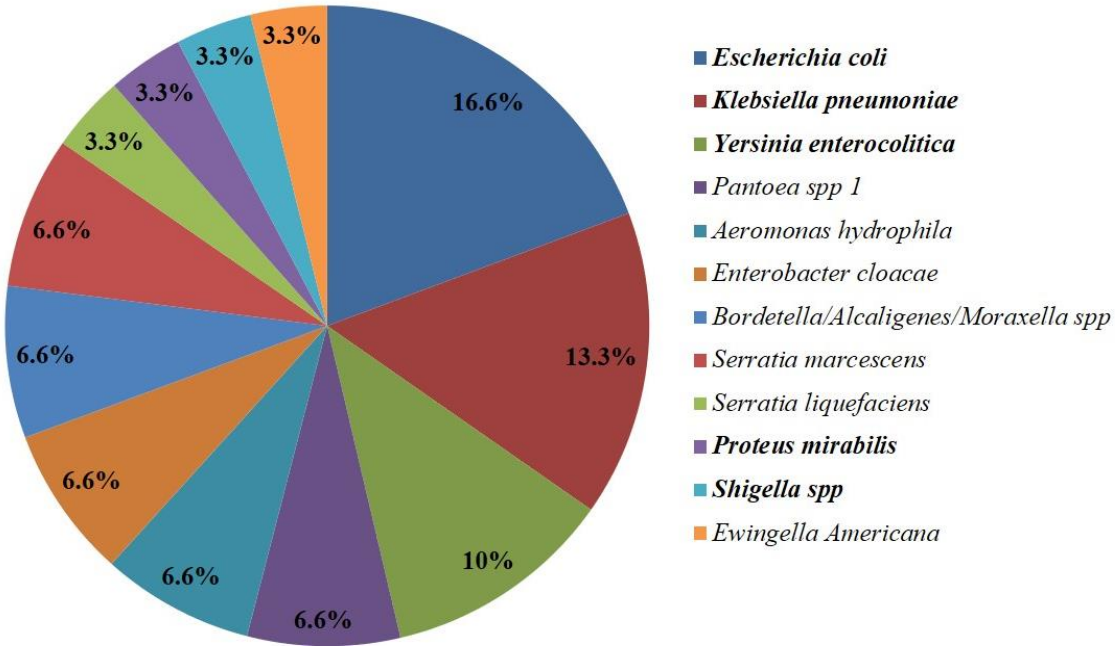

Figure 4. Contamination level of pathogenic coliforms and enteric bacteria for Cilok, dumplings, and skewer meatballs in Yogyakarta.

strains known to be the most abundant facultative anaerobic bacteria in the human digestive tract. Enterotoxigenic *E. coli* (ETEC) was one of the most common causes of diarrhea in children in developing countries, and had six intestinal pathotypes, such as Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* and, enteroinvasive *E. coli*, (including the *Shigella* strain) [24,25,26]. *Yersinia, Shigella, E. coli* producing Shiga toxin was a group of bacteria from enteric pathogens that commonly cause acute diarrhea in the US. The Center for Diseases Control and Prevention estimated that around 1,177,000 yersiniosis cases occurring each year in the US were caused by *Y.enterocolitica* infection, while 90% of them were foodborne through milk, meat, poultry, fruits, vegetables, boiled and fermented products, and seafood [27,28]. *Klebsiella pneumoniae* was a bacterial contamination that ranked third of all the bacteria found in snack food sold in Yogyakarta City, and were found to naturally colonize and live tame in the digestive tracts of healthy animals and humans. However, they were opportunistic pathogens and closely related to foodborne disease outbreaks, especially from retail meats and vegetables. *Klebsiella pneumoniae* was known to cause extraintestinal infections in humans, such as pneumonia, cystitis, pyelonephritis, septicemia, and pyogenic liver abscess. Meanwhile, other groups of bacteria found in this study were generally normal flora and also capable of being an opportunistic pathogens [25,29]. Based on the amount of bacterial contamination in almost all snack food, which had exceeded the threshold limit of the National Agency of Drug and Food Control (BPOM), the safety level of snack food sold in Yogyakatya City needed to be given serious attention, since it promoted health problems in the community.

4. CONCLUSION

The results of Coliform bacterial contamination testing on favorite snack food in Yogyakarta showed that all samples were contaminated by Coliform and most of them exceeded the threshold limit set by the National Agency of Drug and Food Control (BPOM). Furthermore, they were also found to be infected by pathogenic bacteria, such as *Yersinia enterolitica* and

Shigella spp. The amount of coliform contamination in the skewer meatballs was found to be more than 9 log CFU/gr, while cilok and siomay were 2-8 log CFU/gr and 2-7log CFU/gr, respectively. The types of coliforms and enteric pathogens found in snacks food with their contamination levels were namely, *Escherichia coli* (16.6%), *Klebsiella pneumoniae* (13.3%), *Yersinia enterocolitica* (13.3%), *Pantoea spp* (6.6%), *Aeromonas hydrophila.* (6.6%), *Enterobacter cloacae* (6.6%), *Serratia marcescens* (6.6%), *Bordetella / Alcaligenes / Moraxella spp* (6.6%), *Serratia liquefaciens* (3.3%), *Proteus mirabilis* (3.3%), *Shigella spp* (3.3%), and *Ewingella Americana* (3.3%).

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