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ISOLATION AND IDENTIFICATION OF *Klebsiella pneumoniae* IN STREET FOODS AND DRINKS IN YOGYAKARTA, INDONESIA

TRI YAHYA BUDIARSO*, GURUH PRIHATMO, RATIH RESTIANI
and SUHENDRA PAKPAHAN

Biology Department, Faculty of Biotechnology, Universitas Kristen Duta Wacana,
Jl Dr. Wahidin Sudirohusodo 5-25 Yogyakarta, 55224, Indonesia
**E-mail: yahya@staff.ukdw.ac.id*

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ABSTRACT

Healthy foods do not contain harmful ingredients that tend to disrupt the body system, since they are not exposed to bacterial infections, in contrast to street foods which are very vulnerable to bacterial contamination, making them dangerous for consumption. The presence of *Klebsiella* spp. in street foods cause several human infections, such as Urinary Tract Infections (UTI), pneumonia, septicemia, meningitis, rhinoscleroma, ozaena, sinusitis, otitis, enteritis, appendicitis, and cholecystitis. Therefore, this study aims to determine bacterial contamination specifically by *K. pneumoniae* in street foods and drinks. A total of 120 samples were collected from schools and public places often visited by people in Yogyakarta, then processed and subjected to microbiological analysis. From the result, the typical red colonies indicated the presence of *Klebsiella* on the CCA medium and confirmed using the API-20E kit. It was also observed that 11 samples were contaminated with *K. pneumoniae,* while 3 samples were identified with *K. oxytoca,* which demonstrated the presence of bacteria in street foods and drinks. Therefore, government and the Indonesian Agency for Drug and Food Control (BPOM) are encouraged to actively disseminate the benefits of healthy food and provide adequate supervision of food vendors.

Key words: *Klebsiella pneumoniae*, street foods, drinks, API-20E, Yogyakarta

INTRODUCTION

Healthy foods are typically sterile, nutritious, safe, and do not contain harmful ingredients that tend to disrupt the body, since they are not exposed to bacterial infections, in contrast to street foods which are very vulnerable to bacterial contamination making them dangerous for consumption. The *Klebsiella pneumoniae* is known to be pathogenic bacteria, that contaminates food during the processing and packaging stage, therefore, practicing good hygiene towards raw food materials and utensils are important features of food vendors. In addition, environmental sanitation, personal hygiene, and immunity are some of the decisive factors influencing the bacterial infection (Gasink *et al*., 2009).

Klebsiella pneumoniae is a gram-negative bacteria with a short size and rod shape. They are opportunistic pathogen found in the mouth, skin, intestines, hospitals, and medical equipment (Podschun & Ullmann, 1998; Nordmann *et al*., 2011). Previous studies showed that *Klebsiella* cause several infections in humans, including Urinary Tract Infections (UTI), pneumonia, septicemia, meningitis, rhinoscleroma, ozaena, sinusitis, otitis, enteritis, appendicitis, and cholecystitis (CDC, 2009). Urinary tract infection is one of the highest cases of bacterial infection recorded in communities and hospitals (Hryniewicz *et al*., 2001). *Klebsiella pneumoniae* produces biofilms, a matrix of Extracellular Polymer Substances (EPS) that basically consist of poly-saccharides, proteins, lipids, and nucleic acids in various amounts. This matrix resists the effect of antibiotics. Previous studies showed that 65% of hospital-acquired infections are caused by the strain producing biofilms which are 10–1000 times more resistant to the effects of antimicrobial agents and antibiotics (Hoyle *et al*., 1990; Del Papa *et al*., 2007). At least 78 capsular serotypes (K antigens) have been identified as *K. pneumoniae*, which is hypervirulent, very invasive, and cause life-threatening infections in the society, such as

* To whom correspondence should be addressed.

pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis, and severe pneumonia (Shon & Russo, 2012; Shon *et al*., 2013).

Street foods and drinks sold at schools in Yogyakarta (known as education city in Indonesia) are usually inexpensive and come as fast-foods. Meanwhile, it is necessary to test for the food safety and its health impacts on consumers, therefore, this study aims to determine bacterial contamination specifically by *K. pneumoniae* in food and drinks sold at schools and public places in Yogyakarta.

MATERIALS AND METHODS

Collection of bacteria

Yogyakarta is known to be students' city with 2,751 schools and 106 colleges with more than 350.000 students. A total of 120 street food and drink samples were collected from schools and public places often visited in Yogyakarta city, from March 2018 to May 2019. These samples included skewers meatballs, syringes, *cilok*, processed egg, and potato products, as well as packaged beverage products, which are both homemade and industrial product. The samples were placed in separate sterile plastics and stored in an ice packs cooling-box, and transported immediately to Duta Wacana Christian University's Microbiology Laboratory for isolation and identification following the standard method.

Isolation of bacteria

25 grams or 25 ml of each sample was taken and inoculated in 225 ml of buffered peptone water (BPW, Merck, Darmstadt Germany). Then, incubated at 37°C overnight (Guo *et al*., 2016; Wong, 2018). 1 ml of cell culture was diluted with 9 ml peptone water, then homogenized and placed on sterile chromocult coliform agar medium (CCA, Merck Germany). This medium contains Salmon-GAL substrate and X-Glucuronide, which supports the growth of bacteria belonging to the Enterobacteriaceae family, which were further distinguished based on their colonies' appearance. Bacterial gene with β-galactosidase enzymes, grew well on Salmon-GAL substrates, and developed to form salmon-red bacterial colonies, which included, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. Meanwhile, the genus, that was unable to survive the Salmon-Gal substrate made use of β-glucuronidase and X-Glucuronide substrate to give bright blue colonies. The positive groups (β-galactosidase and β-glucuronidase) gave dark blue bacterial colonies namely, *Escherichia* spp. These colonies were distinguished from those of *Klebsiella* spp. using the CCA medium (Turner *et al*., 2000; Manafi, 2003; Rattanabumrung *et al*., 2012).

Selection of *Klebsiella* spp.

Typical red colonies of *Klebsiella* that grew on CCA medium were then scratched to obtain pure isolates and selected through a series of manual tests based on their biochemical properties according to Bergeys' theory used in the identification of *Klebsiella*, *Citrobacter*, and *Enterobacter* isolates. The biochemical tests used in the identification of *Klebsiella*'s isolates included, gram staining, motility, indole, red methyl, Voges-Proskauer, citrate, urease, carbohydrate fermentation, and H_2S fermentation test. During the process, they were all incubated at 37°C for 24–48 hours (Cappuccino & Welsh, 2017; Brenner *et al*., 2015; Patel *et al*., 2017).

Identification of *Klebsiella* using API 20E

The confirmation phase was carried out to identify the isolated bacterial using API 20E and performed according to the French manufactural company's standard (BioMarieux). The pure *Klebsiellas*' isolates were first grown in a Brain Heart Infusion Agar (BHIA) medium for 18–24 hours at 37°C. The Cell culture was then taken using an inoculation loop and dissolved in buffered phosphate saline (5 ml of 0.85% NaCl). The turbidity level of cell suspension was standardized using McFarland 0.5 solution, and dropped asceptically into 20 dishes using a sterile Pasteur pipette. Aquadest was placed at the dish bottom to maintain the moisture level when incubated. The ADH, LDC, ODC H_2S, and URE dish had an underlined code, meaning that their suspension solution was added with mineral oil to half the height of the cupule. The resulting API 20E strip was then incubated at 37°C for 24 hours. Significant changes were observed from the mixtures of IND dish and one drop of James reagent, TDA dish with one drop of TDA reagent, and VP dish with one drop of VP1 and VP2 reagent. Besides, NO_2 test was carried out on GLU dish with the addition of Nit1 and Nit2, while the negative test or the yellow-colored test were performed by adding Zn powder. The results obtained were confirmed using web API software biomereoux (Al-Agha *et al*., 2017).

RESULTS AND DISCUSSION

Isolation and selection of *Klebsiella* spp. from foods and drinks

There were a total of 120 foods and beverage drinks samples used in determining the presence of *Klebsiella* spp. in the CCA medium. The Chromocult Coliform Agar (CCA) medium is a selective media also used for detecting Coliform and *E. coli* bacteria. This media contains peptone,

sodium pyruvate, sodium chloride, sodium dihydrogen phosphate, sorbitol, glued, and chromogenic (Salmon-GAL and X-Glucoronied). Tergitol inhibited the growth of Gram-positive and some Gram-negative bacteria with the exception of Coliform bacteria. However, Pepton, sodium pyruvate, disodium hydrogen phosphate, sodium dihydrogen phosphate, and sorbitol accelerated the Coliform's growth (Turner *et al*., 2000; Lange *et al*., 2013).

Chromocult Coliform Agar was chosen as a medium for isolation, since it distinguishes the genus *Klebsiella* spp. from other genera based on the appearance of their colonies. This medium contained two chromogenic substrates designed for detecting coliform and *E. coli* bacteria with a sensitivity of 91% and 94% and specificities of 94% and 97% (Lange *et al*., 2013). The chromogenic substrate contents included, the enzyme β-glucuronidase (5-bromo-4-chloro-3-indoxyl-bD-glucuronide) commonly called X-GLUC or BCIG having a high specificity (96%) for *E. coli* and the enzyme β-galactosidase (6-chloro-3-indoxyl-bD-galactoside) also known as Salmon-GAL (Lange *et al*., 2013; Turner *et al*., 2000).

Klebsiella spp. is a genus of coliform bacteria, distinguished from other genera in the Enterobacteriaceae family based on their ability to use substrates on CCA medium. Family Enterobacteriaceae has several other genera belonging to the coliform group, namely *Enterobacter* spp. and *Citrobacter* spp. which synthesizes the β-galactosidase enzyme giving the appearance of red or mauve salmon colonies. Meanwhile, *Escherichia coli* consisted of β-glucuronidase and β-galactosidase enzymes and gives the appearance of dark blue or violet colonies on CCA medium (Brenner *et al*., 2015; Lange *et al*., 2013; Teramura *et al*., 2017). The non-coliform bacteria groups that do not possess β-galactosidase enzyme, although, expressed β-glucuronidase enzyme were namely, *Salmonella* spp., *Shigella* spp. and *Yersinia* spp. gave a light blue or turquoise colonies. Furthermore, the non-coliform that do not possess β-galactosidase and β-D-glucuronide produced white or clear yellow bacteria colonies, and do not reduce salmon-GAL into chromogenic compounds, as well as X-glucuronide into X and Glucoronide (Turner *et al*., 2000; Antunes *et al*., 2018). Based on the difference in character, the *Klebsiella* colony that has grown on CCA medium were isolated from other colonies for purification process.

Identification of *Klebsiella* spp.

The pure isolates were then tested for biochemicals to ensure they correspond with the genus *Klebsiella* spp. and subsequently confirmed using API 20E (Figure 1) (Al-Agha *et al*., 2017). The result showed that 11 samples were contaminated with *K. pneumoniae* and 3 with *K. oxytoca*. The API 20E is a biochemical identification kit that is capable of identifying more than 550 different bacterial species. The results of identification showed that the samples were positive for *K. pneumoniae* with the percentage of 85–99% (Table 1). The sampling survey of street food showed the widespread distribution of *Klebsiella* spp. in 5 types of samples, namely ice of cincau, skewered meatballs, traditional milk products, cilok, and skewered eggs, with the exception of milk packaging products. These contaminations were traced to the raw materials, processing equipment, and those processing them.

Recently, the food processing technology has been developed, however, many street food and drinks were still processed traditionally in Yogyakarta. The term street food is related to junk and fast food, since it is part of snacks. The Indonesian Agency for Drug and Food Control (BPOM) and the Directorate of Inspection had supervised various types of street foods from 2008–

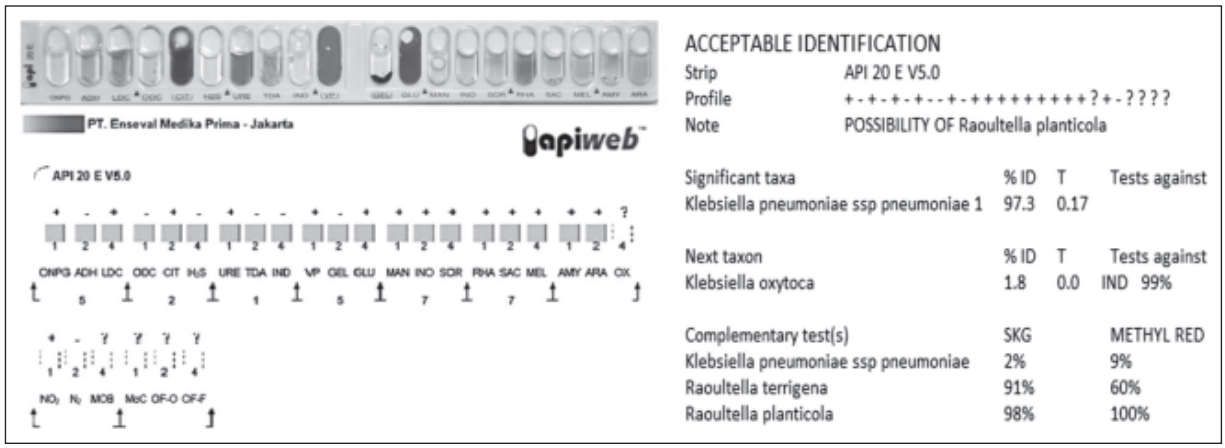

Fig. 1. API 20E test showing the seven-digit number according to the positive results of *Klebsiella penumoniae* 97.3% (Isolate: S10M2).

Table 1. Identification of bacteria using API-20E kit

No	Food Samples	Number of samples	Number of contamination	Code of Samples	Bacteria *Spp.* species
1	Milk packaging	20	3	S13.2 MM S13.102 MU S10.6 PU	*Klebsiella oxytoca* (% ID 97,8%) *Klebsiella oxytoca* (% ID 97,8%) *Klebsiella oxytoca* (% ID 97,8%)
2	Ice Cincau	10	2	SCIN3P4 SCIN2M1	*Klebsiella pneumoniae* (% ID 97,3) *Klebsiella pneumoniae* (% ID 96,9)
3	Skewered meatballs	10	2	S3M1 S10M2	*Klebsiella pneumoniae* (% ID 97,3) *Klebsiella pneumoniae* (% ID 97,3)
4	Traditional milk products	10	4	S_4MM_{10} S_6MM_3 S_8MM_5 S_8MM_3	*Klebsiella pneumoniae* (% ID 85,4) *Klebsiella pneumoniae* (% ID 97,1) *Klebsiella pneumoniae* (% ID 86,1) *Klebsiella pneumoniae* (% ID 85,4)
5	Cilok	10	2	S2M1a S5MG1a	*Klebsiella pneumoniae* (% ID 95,2) *Klebsiella pneumoniae* (% ID 99)
6	Skewered eggs	10	1	S5.1M1	*Klebsiella pneumoniae* (% ID 97,3)
7	Siomay	10	0	–	–
8	Bottled drinking water	20	0	–	–
9	Bottled drinking tea	10	0	–	–
10	Processed potato products	10	0	–	–

2010 and found that 40–44% of street food did not meet the food safety requirements, since they contained hazardous chemicals and ingredients, food additives (BTP), and excess cyclamate and benzoate. Iced-drinks, colored-drinks and syrups, meatballs, and jelly/gelatin are four street food that did not meet the food safety requirements. Coliform bacteria spread through an oral-fecal pathway, by eating food or drink contaminated with human or animal feces through the media of water, hands, or flies. Clinically, common infections are usually caused by *E. coli* and also other coliform pathogenic bacteria, such as *Klebsiella* spp., *Salmonella* spp. and *Shigella* spp. (Batt, 2014). Carrie *et al*. (2018) investigated 1859 cases of meningitis in children under the age of 1 year, meanwhile, 13 *Klebsiella* spp. meningitis cases were registered in the French national registry. *Klebsiella* allegedly cause prematurity, low birth weight, and congenital anomalies of the urinary tract, therefore, bacterial infection is very dangerous for children's growth.

CONCLUSION

The conclusion was supported with the evidence that street foods and drinks sold at schools in Yogyakarta are not safe for consumption, since they are contaminated with *K. pneumoniae*, which is capable of disrupting human health. These bacterial cause diseases, such as the urinary tract infection, septicemia, tissue bronchopneumonia, and gram-negative bacterial pneumonia. Consequently, food vendors are sensitizing to focus more on food sanitation both in the processing and the serving. From the identification of the 120 samples using API 20E, 11 samples were found contaminated with *K. pneumoniae,* while 3 samples with *K. oxytoca*. Therefore, the government and the Indonesian Agency for Drug and Food Control (BPOM) are encouraged to actively disseminate the importance of healthy food and provide adequate supervision of food vendors.

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