Identification of Enteric Bacterial Pathogens in Beverages Sold By Hawkers around Jatinangor, Bandung

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Abstract

Background: Water is essential for life and is considered as a medium for the propagation and dissemination of bacteria. Water-borne disease is one of the problems in Indonesia, therefore, this study was conducted to explore the presence of enteric bacterial pathogens in the beverages sold by hawkers.

Methods: A descriptive laboratory method was conducted in September 2015 Beverages were collected from hawkers that were randomly chosen around Jatinangor using sterile containers, and brought to the laboratory within 1 hour. The beverages were incubated on Mueller Hinton Broth, followed by culturing on MacConkey Agar. The organisms were identified using Gram Staining, Kliger Test, Motility-Indole-Urease test and Citrate test.

Results: Out of 30 samples collected, 2 of the samples were tested positive for enteric bacterial pathogen *Salmonella paratyphi*. Furthermore, *Klebsiella pneumoniae* (n=12), *Enterobacterspp* (n=10) *Alcaligenes faecalis* (n=3) and *Pseudomonas spp.* (n=3) were detected in the samples.

Conclusions: The *Salmonella paratyphi* as enteric bacterial pathogen found in the beverages sold by hawkers has potential to cause water-borne disease. Education to hawkers need to be enhanced.

Keywords: Beverages, Enteric Bacterial Pathogens, Hawkers, *Salmonella paratyphi*

Introduction

Water is indisputably essential in life. All of our body systems depend highly on water, such as in carrying nutrients, eliminating toxin from vital organ and for crucial cellular homeostasis.[1] Nonetheless, it is also considered as a primary medium for the propagation and dissemination of bacteria. Therefore, ingestion of water contaminated by opportunistic pathogenic environmental bacteria may ensue in some serious health implications of waterborne disease. Reports have shown that the incidence of waterborne diseases caused by microorganisms, including the common diarrhea-caused by *Enterobacteriaceae,* has significantly increased over the past few decades.[2] Safe drinking water has then become one of our primary fundamental right ever since. Thus, the importance of prevention against microbial contamination in drinking water is substantially emphasized.[3]

A report of WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation 2012 has been introduced to highlight water supply, sanitation, and hygiene development as its main focuses.[4] Based on the report, Indonesia has over 110 million of the population that has left without proper sanitation and nearly 63 million are still practicing open defecation.[5] Furthermore, about 43 million of people fending without an access to clean water supplies. Consequently, it led to a surge in cases of water-related diseases and worst, some may even result in mortality.[6]

This study was conducted with the purpose of improving the quality of life and saving lives in the near future. Hence, the primary aim of the study was to detect the presence of enteric bacterial pathogens in the beverages sold by hawkers around Jatinangor.

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Methods

This descriptive study was carried out in the Microbiology Laboratory of Faculty of Medicine from September to November 2015. A total of 30 samples were collected from randomly chosen hawkers around Jatinangor, who did not keep the beverages in the fridge. Beverages were defined as drinks made by the hawkersthemshelves. The standard of the beverages was tea with milk, tea with sugar and syrup drink. No ice cubes were present in the drinks. This study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, UniversitasPadjadjaran.

All the samples were collected in clean containers and were brought to the laboratory within an hour. The samples were incubated in Mueller Hinton broth at the temperature of 37ºC for 24 hours. Followed by culture, the samples were then spread on MacConkey agar and incubated in 37ºC for 24 hours. Observations were made to detect growing colonies on the MacConkey. Next, the colonies were collected using a sterile inoculating loop and smeared on the slide for gram staining. These Gram staining procedures used crystal violet solution, iodine solution, alcohol 96% and safranin. The suspected colony was first mixed with NaCl 0.9%. After it became a dry suspension, the suspected colony was mixed with crystal violet solution for 1 minute to stain the cell purple. The slide was rinsed with tap water and poured with iodine solution for 1 minute to color the cells. Subsequently, the slide was rinsed again under tap water and later decolorized by using alcohol 96%. Lastly, safranin was added for another 1 minute. The end result would either show a color of pink or red which indicate the presence of gram negative cells.

Meanwhile, Enteropathogenic bacteria in particular were identified under a microscope using high-powered 100x objective lens with a drop of immersed oil. Following the Gram staining, three more biochemical tests were done using Kliger Iron Agar test (KIA), Motility Indole Urease test (MIU) and the citrate test. Small amount of bacteria colony was streaked in the 3 medium and was incubated in 37ºC for 24 hour. The bacteria were identified by their distinguished appearances according to the specific biochemical test.

Results

Furthermore, the presence of several types of bacteria such as *Klebsiellapneumoniae*, *Enterobacterspp, Alcaligenesisfaecalis, Salmonella paratyphiand Pseudomonas spp,* were also successfully identified (Table 1). Stratification were used to classified bacteria types into pathogenic and non-pathogenic. Pathogenic bacteria, in this study was found

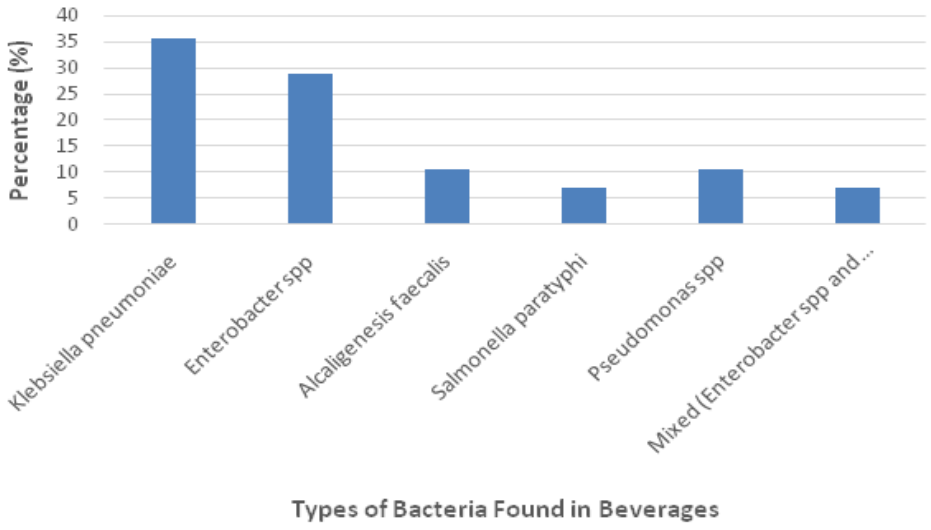

Figure 1 Number of Cases According to Types of Bacteria Found in Beverages

Table 1 Types of Bacteria Present in Beverages Sold by Hawkers around Jatinangor, Bandung

Beverages	Identification Results	Type of Bacteria
Syrup drinks	Negative	-
Tea with milk	Negative	-
Syrup drinks	Negative	*Alcaligenesisfaecalis*
Tea with milk	Negative	*Alcaligenesisfaecalis*
Tea with sugar	Negative	*Alcaligenesisfaecalis*
Syrup drinks	Lactose (+) Mobility (+) Citrate (+)	*Enterobacterspp*
Syrup drinks	Lactose (+) Mobility (+) Citrate (+)	*Enterobacterspp*
Syrup drinks	Lactose (+) Mobility (+) Citrate (+)	*Enterobacterspp*
Syrup drinks	Mobility (+) Citrate (+)	*Enterobacterspp*
Tea with milk	Lactose (+) Mobility (+) Citrate (+)	*Enterobacterspp*
Tea with milk	Mobility (+) Citrate (+)	*Enterobacterspp*
Tea with sugar	Mobility (+) Citrate (+)	*Enterobacterspp*
Tea with sugar	Lactose (+) Mobility (+) Citrate (+)	*Enterobacterspp*
Tea with milk	(A)Mobility (+) Citrate (+) (B) Lactose (+) Citrate (+)	*Enterobacterspp* *Klebsiella pneumoniae*
Tea with sugar	(A) Mobility (+) Citrate (+) (B) Lactose (+) Citrate (+)	*Enterobacterspp* *Klebsiella pneumoniae*
Syrup drinks	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Syrup drinks	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Syrup drinks	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with milk	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with milk	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with milk	Lactose (+) Gas (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with sugar	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with sugar	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with sugar	Lactose (+) Gas (+) Citrate (+)	*Klebsiella pneumoniae*
Tea with sugar	Lactose (+) Citrate (+)	*Klebsiella pneumoniae*
Syrup drinks	Mobility (+) Citrate (+)	*Pseudomonas spp*
Tea with milk	Mobility (+) Citrate (+)	*Pseudomonas spp*
Tea with milk	Mobility (+) Citrate (+)	*Pseudomonas spp*
Tea with sugar	Mobility (+)	*Salmonella paratyphi**
Tea with sugar	Mobility (+)	*Salmonella paratyphi**

Note: ** were pathogenic bacteria

in 2 cases (7.1%) whereas the rest of 26 cases (92.9%) were non-pathogenic bacteria (Table 1) .

As a result, a crucial pattern on the types of bacteria frequently encountered in the beverages, from the least to the commonest, was able to be deduced (Figure 1). The commonest bacteria found were *Klebsiella pneumoniae* (35.7%) followed by *Enterobacterspp* (287%), *Alcaligenesisfaecalis* (10.7%), *Pseudomonas spp* (10.7%) and *Salmonella paratyphi* (7.1%). However, there were also 7.1% of mixed growth between *Enterobacter spp* and *Klebsiellapneumoniae*.

Table 2 Types of Beverages Contaminated with Various Bacteria

Beverages	Type of Bacteria	N
Syrup drinks	None detected	1
	Alcaligenesisfaecalis	1
	Enterobacterspp	4
	Klebsiella pneumoniae	3
	Pseudomonas spp	1
Tea with milk	None detected	1
	Alcaligenesisfaecalis	1
	Enterobacterspp	2
	Enterobacterspp	1
	Klebsiella pneumoniae	3
	Pseudomonas spp	2
Tea with sugar	*Alcaligenesisfaecalis*	1
	Enterobacterspp	2
	Enterobacterspp	1
	Klebsiella pneumoniae	4
	Salmonella paratyphi	2

Discussions

Out of 30 beverages samples collected, 28 samples (93.3%) have been contaminated with either pathogen or non-pathogen bacteria. Such results may depict that hawkers in Jatinangor are majorly serving contaminated beverages to its customers, by change. This intricately reflects that many people havebeen exposed to unwanted, yet serious health implications like waterborne diseases, after ingesting those contaminated drinks.[7]

Similarly, study in Pakistan[8] has shown that there is a high amount of microbial contamination found in the drinks sold by street hawkers. Unhygienic environment and poor handling practices definitely have a strong association to the microbial contamination found in the drinks. These reports also show the possibility of bacteria to be able to survive in drinks especially within the low economic areas. [8]

In addition, this study is able to deduce the commonest bacteria found within beverages. Klebsiella pneumoniae was the most common bacteria found in baverages (35.7%), despite their non-pathogenic properties in gastro-intestinal tract. This may be because of their non-pathogenic character. *Klebsiella spp* can be easily found in the respiratory tract and human feces, but they could only pose threats when they are displaced from their normal habitat. Therefore, *Klebsiella spp* plays only a minimal role in causing waterborne disease.[9]

Whilst, the second most *Enterobacteriaceae* that have been isolated from the beverages was *Enterobacter spp*. Several *Enterobacter spp* are known as opportunistic pathogens in human that can be found mostly on human skin and in the intestinal tract. This bacteria can barely survive in low pH environment, hence the secretion of gastric acid plays an important role in killing the bacteria.[10] Thus, *Enterobacter spp* poses even a lower risk to cause harm to humans.

Intricately, the worst pathogen found within the beverages were *Salmonella paratyphi,* mostly found in animal feces. The bacteria had indeed known to be pathogenic towards human, in which one may suffer severe fever and abdominal pain. *Salmonella paratyphi* possess several unique characteristics that enable them to survive harsh environments, such as the ability to grow at 7–48°C with an optimum growth at human's body temperature of 37°C and at pH of 4 to 9.5 with an optimal growth at pH 6.5 to 7.5.11 Therefore, *Salmonella spp* manage to survive in the stomach through the induction of acid

tolerance response and the ability to undergo an adaptive response to moderately acidic pH.[12] The short emptying time after consume beverage could also facilitate Salmonella sppsurvival.[13]

The presence of enteropathogenic bacteria such as Salmonella paratyphiis correlated with poor personal hygiene and environmental sanitation, lack of supply of safe water and ignorance of health promotion practices. These may indirectly due to low education status which may lead to outbreaks of infectious diseases among the population. Other species of *Enterobacteriaceae* that detected is supporting the idea of contamination of fecal bacteria due to inadequate hand washing of the hawkers. The non-enterobacteriaceae that detected is commonly presence in environment such as soil and water. Relatively, indicative of poor hygiene when handling food and beverages.

This study was conducted within 3 months, which limited further collection, culturing and analyzing for even bigger sample size. With only 30 samples in total, this study was therefore insufficient to conclude that the enteric bacteria pathogen, especially *Salmonella paratyphi* is commonly present in beverages sold by hawkers, to be said potentially causing waterborne diseases. Moreover, the shortage of budget at hand was nearly inadequate to cover the total cost of all the microbiology examinations, especially the agar. Despite all the limitations stated, this study is still able to detect numbers of beverages containing pathogenic enteric bacteria.

In conclusion, beverages sold by hawkers possess a potential risk to cause serious health implications like waterborne diseases, especially in the presence of Salmonella paratyphi. A proper handling during beverages preparation as well as environmental sanitation are the vital factors to help in preventing further risks to consumers, thus, improving the quality of life for humans.

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