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Short Communication

Enumeration of *Escherichia coli* from fresh coconut milk in Negeri Sembilan, West Malaysia

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Abstract

Escherichia coli is commonly found in the intestinal tract of human and warm-blooded animal. Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7 are pathogenic and able to cause serious health problem to human. In this study, the detection of *E. coli* from raw coconut milk was carried out by using the most-probable-number (MPN) and streaked plate methods. A total of 125 samples were purchased randomly from five hypermarkets and 16 wet markets in Kuala Pilah, Senawang and Seremban, West Malaysia areas. The samples that contaminate with *E. coli* were found to be ranging from $<3.0x10^4$ MPN/ml to $>2.4x10^7$ MPN/ml. The results revealed the presence of *E. coli* in fresh coconut milk from wet markets and hypermarkets in Negeri Sembilan. Thus, the result showed high health risk and the need for improving hygienic standard among food handlers. Therefore, public should be aware and always practice proper food handling.

Introduction

Pathogenic *Escherichia coli* strains are categorized into six specific groups or pathotypes based on their virulence determinants. Enterohaemorrhagic *E. coli* (EHEC) is considered as a subset of Shiga toxin-producing *E. coli* (STEC) and it is also named as Verocytotoxic-producing *E. coli* (Otero *et al.*, 2014). Shiga toxin-producing *E. coli* (STEC) are the important emerging pathogens which cause foodborne infection. They are able to adhere to the epithelial cell of the gastrointestinal tract and cause bloody diarrhea. The severe diarrhea may develop into hemolytic uraemic syndrome (HUS) (Loo *et al*., 2013).

Escherichia coli O157:H7 serotype is a gram negative bacteria and pathogenic to human. They have a rod shape and associated to the virulence genes of the Shiga toxin 1 *(stx1)* and Shiga toxin 2 *(stx2)* or combinations of both (Suria *et al.*, 2013). Haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) are two serious human life-threatening diseases caused by the release of virulence genes Shiga toxins (*stx1* and *stx2*) (Kamal *et al*., 2014).

Escherichia coli O157:H7 was first considered as a pathogen during an outbreak investigation of hemorrhagic colitis in 1982 (Lye *et al*., 2013). As reported by Derzelle *et al.* (2011) a large outbreak caused by STEC O104:H4 had occurred in the European Union (EU), with 3959 cases including 43 deaths. The latest outbreak of *E. coli* O157:H7 was reported in July 2014 in the United States (US) with 17 persons infected (CDC, 2014). Therefore, *E. coli* O157:H7 has been recognized as one of the most significant foodborne pathogen relating to the public health especially in South Africa, Europe, Japan and United States (US) (Jeshveen *et al*., 2012).

Aslam *et al.* (2003) reported that the most common food-borne pathogen found in raw milk was Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella*. However, the pathogenic *E. coli* that has greatest relevance to milk is *E. coli* O157:H7, a STEC serotype and also as one of major concern in the dairy industry (Farrokh *et al.*, 2013). This study was undertaken to determine the presence of *Escherichia coli* in raw and unprocessed coconut milk isolated from wet markets and hypermarkets in Kuala Pilah, Senawang and Seremban. The study is important since fresh coconut milk are being used in the preparation of foods in Malaysia such as 'cendol'. It can create awareness among public especially housewife and food handlers towards the danger of bacteria such as *E. coli.*

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Materials and Method

Sampling

A total of 125 samples of fresh coconut milk were purchased randomly from five hypermarkets and 16 wet markets in Kuala Pilah, Senawang and Seremban, West Malaysia areas. The samples from different markets were collected in sterile plastic bag and put into ice box before transported to the laboratory. All samples were kept at 4°C in the laboratory within the six hours period until further analysis. All equipment used were sterilized to avoid cross contamination during analysis.

Isolation of Escherichia coli

A 10 ml of each sample was placed in a sterile stomacher bag together with 90 ml of nutrient broth (NB) and then were homogenized for 30 seconds. Later, the homogenized samples were incubated at 37°C for 24 hours. After that the incubated samples then furthered for the MPN three tubes method.

Most-probable-number (MPN) and streak plate method

The three tubes MPN method was carried out by preparing a serial dilution from 10^{-1} to 10^{-7}. One ml of the samples were inoculated into the broth with ratio 1:10. One ml of the aliquot from 10^{-5}, 10^{-6} and 10^{-7} were transferred in triplicates MPN tubes and incubated at 37°C for 24 hours. The turbid MPN tubes were then streaked on the CHROMagar *E. coli* (CHROMagar, Paris). Expected *E. coli* with blue colonies were picked after 18-24 hours of incubation. The expected *E. coli* colonies were then grew on nutrient agar (NA) slant (Merck, Germany).

Results and Discussion

The estimated quantity of *E. coli* from Kuala Pilah samples was ranging from <3.0x104 MPN/ml to >2.4x10^7 MPN/ml, Senawang samples from <3.0x10^4 MPN/ml to 1.1x10^7 MPN/ml and Seremban samples was <3.0x10^4 MPN/ml (Table 1). In general the most probable number of *E. coli* in the wet market samples varied from <3.0x10^4 MPN/ml to >2.4x10^7 MPN/ml while in the hypermarket samples varied from <3.0x10^4 MPN/ml to 1.2x10^6 MPN/ml. The quantity of *E. coli* is higher in the wet market samples compared to the hypermarket. On the other hand, the highest quantity of *E. coli* was collected in Kuala Pilah area with >2.4x10^7 MPN/ml and the lowest quantity was collected in Seremban area with <3.0x10^4 MPN/ml.

Shiga like toxin *E. coli* (STEC) can cause hemorrhagic colitis (HC) and hemolytic uraemic syndrome (HUS) and it has become a serious health problem in various countries (Rey *et al.*, 2006). *Escherichia coli* was generally found in all samples in a range of <3.0x10^4 MPN/ml to >2.4x10^7 MPN/ml. This result showed that there was a broad range of the *E. coli* contaminated the fresh coconut milk. Therefore, several steps should be taken to avoid the infection from this food-borne pathogen.

The highest MPN value of *E. coli* was found to be in the wet markets of Kuala Pilah (>2.4x10^7 MPN/ml) while there is just a few contamination of *E. coli* at other wet markets. As observed, the machine used to process the coconut milk in Kuala Pilah wet markets were kept unhygienically and the tapped water was used to process the coconut milk. These unhygienic practices resulted in high chances for the coconut milk to be contaminated. In May 2014, a warning was sent to people in Portland, Ore that they should boil all tap water used for drinking, food preparation, tooth brushing and ice for at least one minute due to the presence of *E. coli* in the samples taken from the routine drinking water (NBC News, 2014). While observing at the hypermarket level, the prevalence of *E. coli* was low because the coconut milks were stored at the good hygienic condition. Most of the coconut milks in the hypermarket were properly packed and stored at 4°C.

Cross contamination could be the reason of why *E. coli* was highly present in the samples from wet market even though the coconut milk was covered with ice to maintain its freshness. Other than that, improper handling of the food products can be considered as one of the major factors contribute to contamination of food at the sampling areas. According to Puspanadan *et al*. (2012) poor hygienic practices may also contribute to the contamination of foods.

Akbar *et al.* (2012) reported that the presence of *E. coli* in food material is considered as an indicator for another pathogenic bacteria in the respective food items. *Escherichia coli* should not be found in any samples of the raw coconut milk. By observation, the unsterile environment and utensils used can lead to the contamination of *E. coli* in the food samples. There are some strains identified as the serious causal agents of various illness (Samuel *et al*., 2011). Sahilah *et al.* (2010) stated that Shiga toxin-producing *E. coli* (STEC) are among the most important of food-borne disease which are responsible for human gastrointestinal disease.

Another factor which may contribute to the higher growth of bacteria is by mixing of fresh milk with milk of the day before (Hadrya *et al*., 2012).

Table 1. The most-probable-number (MPN/ml) of *Escherichia coli* isolated from raw and unprocessed coconut milk

Area	Wet Market			Hypermarket		
	[a]Min	[b]Med	[c]Max	[a]Min	[b]Med	[c]Max
Kuala Pilah	$<3.0x10^4$	$1.2x10^6$	$>2.4x10^7$	$<3.0x10^4$	$7.5x10^5$	$1.2x10^6$
Senawang	$<3.0x10^4$	$<3.0x10^4$	$1.1x10^7$	$<3.0x10^4$	$<3.0x10^4$	$<3.0x10^4$
Seremban	$<3.0x10^4$	$<3.0x10^4$	$<3.0x10^4$	$<3.0x10^4$	$<3.0x10^4$	$<3.0x10^4$

[a]Min= Minimum MPN/ml value, [b]Med= Median MPN/ml value, [c]Max= Maximum MPN/ml value

Therefore, the high presence of *E. coli* in the coconut milk may be due to the improper processing and the storage condition. According to Sim *et al.* (2012) the source of contamination happened along the milking chain and it mostly due to the unhygienic milking environment and also contaminated milking equipment. The high ambient temperatures also can lead to the growth of bacteria during sales and transport of the milk (Benyagoub *et al*., 2013). At the wet market, some sellers do not have a cold chain in which the temperature surrounding the coconut milk may increase exceeded 25°C. Therefore, it will stimulate the growth of bacteria such as *E. coli.*

In the Food Act 1983 stated that the coconut milk should contain not less than 12.7% and not more than 25.3% of total solid, 2.7% of non-fat solid and 10% of fat. It could have a pH of not less than 5.9, be free from kernel residue and there should be no *E. coli* found in 100 ml of any water. Coconut milk is widely used in the preparation of food in Malaysia. Some of the foods such as 'cendol' use fresh coconut milk in their preparation. Therefore, society especially food handlers and housewives should be more aware about the fresh coconut milk hygiene in order to avoid contamination of the pathogenic bacteria.

Conclusions

This study revealed the presence of *E. coli* in fresh coconut milk obtained from wet markets and hypermarkets in Negeri Sembilan. It is presume that the factors of the contaminated foods are the improper handling during the processing the coconut milk and low hygienic practices among the sellers. Maintaining good hygienic process and the proper handling during processing the coconut milk is important as the pathogenic bacteria may be harmful to the consumers. Housewives and the food handlers should be more aware of the presence of *E. coli* as its can contribute to the serious disease such as hemolytic uraemic syndrome (HUS).

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