

Occurrence of *Escherichia coli* harbouring *stx* genes in popiah, a Malaysian street food

¹*Elexson, N., ²Nik Yuhanis, F.N., ²Malcolm, T.T.H., ²New, C.Y., ²Chang, W.S.,
²Ubong, A., ²Kuan, C.H., ²Loo, Y.Y., ²Thung, T.Y. and ^{2,3}Son, R.

¹Department of Biology Molecule, Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

²Department of Food Science, Faculty of Food Science and Technology,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

³Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security,
Universiti Putra Malaysia 34300 Serdang, Selangor, Malaysia

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Abstract

Irrespective of its health effects, street foods are very popular with the consumers. The main purpose of this research was to study the biosafety of *Escherichia coli* in popiah, a Malaysian street food sold at a roadside food stall and a restaurant in Sri Serdang, Selangor, Malaysia, using the combination of the most probable number (MPN)-Polymerase Chain Reaction (PCR) assay-plating on Eosin Methylene Blue (EMB) agar methods. Using these biomolecular methods, *E. coli* was detected in 12/15 (80%) and 11/15 (73%) of the collected samples from the roadside food stall and the restaurant respectively. The incidence of *stx* virulence-associated genes was detected in 1/15 (7%) among the *E. coli* isolated from samples taken from the roadside food stall while the *E. coli* isolated from the restaurant was 3/15 (20%). The density of *E. coli* ranged from <3 to >1100 MPN/g and the density of *E. coli* positive with *stx* genes was <3 to 53 MPN /g in samples from both the roadside food stall and the restaurant. The presence of the *stx*-positive *E. coli* in popiah are significant to risk assessments of food and epidemiological studies. Therefore, from the information obtained in this study, it is obvious that the screening for STEC markers in food samples would be useful for food safety survey.

1. Introduction

It is now a well-accepted fact that street food vending represents an important food security strategy for low-income communities worldwide. However, more stringent measures must be promoted in street food vending to integrate the prevention of biological risk factors in order to promote comprehensive and up-to-date consumer safety as these foods are most commonly sold in the streets, public places, busy market places, school areas, near college campuses, and taxi stands, etc. Thus, the safety of street foods has been a source of concern to consumers due to its popularity, particularly in relation to their microbiological contamination.

Popiah, is a Malaysian street food favoured by many. It is mostly made with a filling of shredded raw vegetables, and various studies has shown that raw vegetables represent an important source of risk for human health because they are carriers of pathogenic micro-organisms (Harris *et al.*, 2003; Park *et al.*, 2012). The Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic

E. coli (ETEC), Enteroinvasive *E. coli* (EIEC) and non-O157 Shiga toxin-producing *E. coli* are the important causative agents of diarrhea in developed countries (Nataro and Kaper, 1998; Nataro, 2004; Estrada-Garcia *et al.*, 2005, 2009). However, STEC strains, harbouring the *stx* genes is one of the most important recently emerged groups of food borne pathogens, and can contaminate fresh vegetables via primary contamination (while growing and during harvest) or secondary contamination (during washing, slicing, soaking, packaging and preparation) (Harris *et al.*, 2003).

To reduce the exposure of humans to this agent, the detection of *E. coli* based on its recovery from food samples and confirming the presence of its virulence associated factors (shiga-toxins) (Vernozy-Rozand, 1999) is a central goal. Conventional methods are still regarded as the basic tools for isolation, detection, and identification of foodborne pathogens since not all laboratories in developing countries have sufficient resources to incorporate PCR methods in their analytical procedure. The objective of this study was to determine the incidence and density of *E. coli*

*Corresponding author.
Email: nelexson@frst.unimas.my

and *E. coli* harbouring the *stx* genes in popiah using biomolecular method.

2. Materials and methods

2.1 Sampling method

A total of 30 popiah samples were randomly collected directly from a roadside food stall and a restaurant in Sri Serdang, Selangor, Malaysia. The two sampling sites were visited once a week for sample collection. Samples were collected aseptically in pre-sterile poly-bags and, then were transferred to the laboratory for immediate analysis.

2.2 Most probable number count

Ten grams of sample was homogenized with 90 ml of 0.85% (w/v) sterile physiological saline solution in a stomacher laboratory blender (400, Seward, Worthing, UK) for 1 min. The most probable number (MPN) count of *E. coli* was determined following the method of Cappuccino and Sherman (1998). Turbid MPN tubes were further streaked on Eosin Methylene Blue (EMB) agar (M317, HiMedia) plates and incubated at 37°C for 18 - 24 h. Distinctive metallic green sheen colonies were picked and screened for the presence of the *stx* genes Polymerase Chain Reaction (PCR).

2.3 PCR screening for *stx* genes

A well isolated colony was transferred to 2 ml of TSB and incubated overnight in an incubator shaker. A 1 ml portion of the cultures was centrifuged at 15,000 x g for 1 min. The pellet was re-suspended in 500 µl sterile distilled water and vortexed vigorously. The cell suspension was boiled for 10 min, immediately chilled on ice for 10 min and centrifuged again at 15,000 x g for 1 min. The supernatant containing crude DNA was transferred into a new 1.5 ml microcentrifuge tube and 5 µl was used as DNA template in PCR. All DNA samples were screened for the presence of *stx* genes by PCR as described by He *et al.* (2012). The PCR product was analysed by electrophoresis on 2% agarose gel and stained with 0.3 µg/mL ethidium bromide for visualization of the amplicons under UV light gel documentation system (Alpha Imager®, Alpha Innotech).

3. Results and discussion

Popiah is a popular street food in Malaysia and, it is also one of the popular dishes served at home. Most vendors prepare popiah at their homes before being sold in the streets, public places, busy market places, school areas, near college campuses,

and taxi stands, etc. The changes in consumer food behaviours and food patterns have led to an increase in the demand for pre-prepared ready-to-eat (RTE) foods or snacks, such as popiah, and that few data are currently available on the occurrence of *E. coli* in such foods in Malaysia. In fact, minimal studies were conducted on the pathogen prevalence in street foods in most countries. *E. coli* was detected in 12/15 (80%) and 11/15 (73%) of the popiah collected from the roadside food stall and the restaurant, respectively (data not shown). Studying the incidence of *stx* virulence-associated genes revealed the presence of the *stx* genes in 1/15 (7%) and 3/15 (20%) among the *E. coli* isolates tested from popiah samples from the road side stall and the restaurant, respectively (data not shown) and *E. coli* harbouring *stx* genes are presumably known as STEC. The density of *E. coli* ranged from <3 MPN/g to >1100 MPN/g while the density of *E. coli* positive for the *stx* genes was <3 MPN/g to 53 MPN /g in samples from both the roadside food stall and small restaurant (data not shown). The presence of *E. coli* and *E. coli* harbouring *stx* genes is generally related to the low hygiene and sanitation knowledge and practice of the food handlers. It should be noted that microbial population is not well-distributed in food and the transfer of the pathogen from one food to another could presumably due to the same point of contact such as the utensils used, in which will have an increased percentage of transmission when low hygiene and sanitation is being practiced. Several studies were conducted to evaluate the knowledge, attitude and practice of food hygiene and sanitation among the food handlers in Malaysia and the outcome of the studies revealed that Malaysian food handlers had a moderate to high levels (Saad *et al.*, 2013; Mohd. Firdaus Siau *et al.*, 2015). It is possible to rule out this factor with the reported results but what seems to be the problem here is the lack of consistent practice by the food handlers. Furthermore, the complexity of the food supply chain from the initial process to the final process allows many intervention and possible unwanted contaminations of the pathogen.

On the other hand, the shredded raw vegetables, i.e. bean sprouts, used as a filling of the popiah are normally washed with tap water. The water used could be a source of contamination of *E. coli*, if it is poorly treated. In addition, raw vegetables such as bean sprouts may represent an important source of risk for human health because they can become carriers of pathogenic micro-organisms (Harris *et al.*, 2003; Park *et al.*, 2012). Indeed, their complex surface and porosity, unfortunately facilitate the pathogen attachment which leads to their survival

(Report of the Scientific Committee on Food, 2002; Said, 2012). The popiah will be a direct exposure route of the pathogen to humans as this ready-to-eat street food is consumed without further treatment that would eliminate or reduce the microbial load. As STEC is often the top foodborne pathogens, their exponential growth in human guts with only minimal cells can cause foodborne illness with a high likelihood. In our study, it is interesting to note that there was an obvious difference in the occurrence of STEC contamination between the roadside food stall and the restaurant. This fact might be explained by the different environment and management of the roadside food stall and the restaurant. Usually, in small settings as conducted in this study, it is unable to trace the food sale chain. Some stall keepers produced food by themselves while others purchased food from individual or part-time popiah makers, hence the food sale chain is rather complex and has no obvious information for trace back.

Diarrheogenic *E. coli* pathotypes (DEPs) are important foodborne pathogens (Kaper et al., 2004) and are classified per their unique virulence traits: Shiga toxin-producing *E. coli* (STEC); enteroaggregative *E. coli* (EAEC); diffuse adherent *E. coli* (DAEC); enteroinvasive *E. coli* (EIEC); enterotoxigenic *E. coli* (ETEC); enteropathogenic *E. coli* (EPEC); and entero-aggregative-hemorrhagic *E. coli* (EAHEC). Non-STEC strains are mostly commensal bacteria in animals, with a high potential for food-borne transmission to humans (Caprioli et al., 2005), though the majority of non-STEC strains have low virulence. However, a food-borne outbreak involving an EAHEC strain (*E. coli* O104:H4) originating from sprouts in Germany and France (Buchholz et al., 2011), was reported to have acquired the *stx* genes (Muniesa et al., 2012; Grad et al., 2013) illustrates on how the mobile *stx* genes can be transferred to different classes of pathogenic *E. coli*, making them hypervirulent pathogens and having the potential to emerge as new Shiga toxin-producing *E. coli* strains (Muniesa et al., 2012; Grad et al., 2013). This also justifies the purpose of this study to screen for the presence of *stx* genes.

Street foods is the “traditional fast food” of developing countries, as opposed to the growing presence of “factory cooking” or “fast food” and the consumption patterns (frequency and regularity of consumption) vary from country to country and are influenced by the national and/or regional food cultures. The results obtained in our research provided important information on the health risks associated with the direct consumption of street foods. The risks associated with the street foods

can further be controlled and made well-mitigated if all the stakeholders play their roles efficiently (consumers, vendors and government authorities), and be promoted as an alternative in ensuring food security for low-income group urban populations.

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