Bacterial contamination of raw vegetables, vegetable-related water and river water in Ho Chi Minh City, Vietnam

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ABSTRACT

The study attempts to identify the potential routes of bacterial infection via consumption of raw vegetables, drinking water and vegetable-related water in Ho Chi Minh City (HCMC). Vegetables in the markets and restaurants had higher total coliforms and *E. coli* counts than the vegetables at the vegetable cultivation fields. In search of the potential contamination sources, it was found that vegetables are washed in nearby canals after harvesting. Those canals are contaminated with human and animal excreta, which in turn may contaminate the vegetables. At the markets, although the tap water was found to be free of microbes, contaminated and non-contaminated vegetables are mixed and washed in the same bowl, which may bring about further spreading of infectious bacteria. The results of this study suggested that an integrated countermeasure that incorporates reducing microbial contamination of canals, raising the awareness of microbial infection among the local farmers and wholesalers, and providing enough clean water to the food markets should be implemented to reduce the incidence of food-borne illness in HCMC.

**Key words** | *Escherichia coli*, Ho Chi Minh City, raw vegetables, total coliforms, vegetable-related water

INTRODUCTION

Raw fruits and vegetables have been known to serve as vehicles of human disease for more than a century. However, raw fruit and vegetables are an essential part of people’s diet all around the world. Eating raw vegetables is quite traditional and popular in Vietnam and some other Asian countries. About 85% of vegetables in Vietnam are produced by rural households; that is, ca. 10.2 million people are engaged in vegetable production. The vegetable-farming area increased in 2003 by 17,163 ha from 2002, which is equivalent to an annual increasing rate of 3.06%. Total vegetable productivity has also increased at a rate of 8.88% per year between 1998–2003, which corresponds to an increase from 5.24 to 8.18 million tons per year (RIFAV 2004). Vegetable production has also significantly increased because the agricultural practice has become more intensive, which requires more fertilizers, including manure, animal compost or inorganic fertilizers, to be sprayed in the agricultural fields.

In developing countries, continued use of untreated waste water and manure as fertilizers for the production of vegetables is a major contributing factor to bacterial contamination that causes numerous food-borne disease outbreaks (Beuchat 1998). *E. coli* thrives in the intestines of humans and animals. Its particular strain, *E. coli* O157:H7, causes serious illness among people (Feng et al. 2002) and significant economic loss (Frenzen & Drake 2005). Vietnam
has high mortality amongst children younger than 5 years old, i.e. 42.2 deaths per 1,000 capita per year, of which 15% is related to diarrhea disease (Doan et al. 2003). There are about 200 serious cases of bacterial infection per year in HCMC hospitals (Lien 2006). Hong (2007) reported that more than 97% of 104 raw vegetable samples collected in 13 food markets in Ho Chi Minh City (HCMC) were contaminated by either worm larvae, *Entamoeba histolytica* or *Escherichia coli* (*E. coli*), with their contamination rates of 78.8%, 65.4% or 50%, respectively. Another survey conducted in Hanoi in 2004 also found serious contamination by *E. coli* of raw vegetables. The percentage of vegetables with *E. coli* is ranked second at 18.5%, just lower than the percentage of raw meat contaminated with *E. coli* (Dao & Yen 2006). Accordingly, there are growing concerns on the risk of food-borne bacterial diseases due to the oral intake of pathogens via vegetables eaten raw in Vietnam. Additionally, bacterial contamination of canals and rivers in HCMC has reached an alarming level. The indicator bacteria in Saigon River are 3 to 168 times higher than the Vietnamese Environmental Standards between 2001 and 2006 (HEPA 2006). This situation has been brought about through the rapid urbanization and industrial development in recent years, combined with the slow rate of infrastructure construction, poor awareness of environmental protection and lack of political mechanisms for the water environmental management in HCMC (Toan 2006).

These facts suggested that there exists a risk of bacteria transmission via fecal-oral route due to bacterial contamination of environmental water and raw vegetables in and around HCMC. The objective of this study is to elucidate the microbial transmission pathways by measuring bacterial indicators, *E. coli* and total coliforms (TC) in the contaminated raw vegetables, vegetable-related water and river water in HCMC.

### MATERIALS AND METHODS

#### Study area

HCMC is known as the most active and dynamic economic center in Southern Vietnam. HCMC covers about 2,095 km² including 19 central districts and 5 sub-urban districts. The population is about 6.2 million; the urban population density is 10,608 persons per square km (http://www.pso.hochiminhcit.gov.vn); the average annual income in 2006 is US$ 876 per capita; the annual fruit-vegetable consumption is 71 kg per capita (RIFAV 2004). The total area producing clean and safe vegetables (CSV) is ca. 2,100 ha, comprising of Cu Chi, Hoc Mon, Go Vap, Thu Duc suburban districts, which supplies only 30% of CSV demands for HCMC (Anh 2005). Especially, Hoc Mon District is a center of vegetable production and delivery with a vegetable wholesale market, named Moi Hoc Mon, where all raw vegetables are transferred from farmlands before delivering to the retail markets in the city such as Pham Van Hai, Tan Binh, and Cau Muoi Markets. In addition, Da Lat Market, located in Lam Dong mountainous Province, about 500 km away from HCMC, supplies most of the vegetables to HCMC (Figure 1).

HCMC is characterized by an interlacing river-canal system. Saigon (SG) River is the second largest river after Dong Nai River that run through HCMC. The total length of SG River is 280 km and the catchment is about 5,000 km². It originates from Tay Ninh Province, runs through Dau Tieng Reservoir to downstream, from Northwest to Southeast, dividing Binh Duong and Tay Ninh Provinces and flows through HCMC before the confluence with Dong Nai River at Nha Be, where it is defined as the SG river mouth at 0 km. Ben Than Water Intake is located at about 55 km upstream from SG river mouth and supplies about 300,000 m³ water per day to HCMC. The canal system in HCMC has a total length of 93 km, and is a natural drainage scheme for all sewage and run-off from the city and the upstream provinces. The HCMC Environmental Protection Agency (HEPA) reported that about 150,000 m³ of industrial waste water; 17,000 m³ of hospital waste water; 500,000 m³ of domestic waste water; 400–500 tons of solid waste; and 300 tons of human wastes are disposed directly to HCMC canals everyday with/without preliminary treatment (HEPA 2006).

#### Samplings

This study focused on the north and central parts of HCMC where there are large-scale vegetable fields and...
the places to store, wash, deliver and sell raw vegetables. In total, thirty-six raw vegetable samples were collected in September 2006 and in January 2007. In term of vegetable species, our study focused only on two types of vegetables such as mint (*Ocimum tenuiflorum* L.) and salad (*Lactuca sativa* L.), which are commonly eaten raw in Vietnam. The vegetable-sample set comprised 21 samples taken at 5 food markets (Da Lat, Hoc Mon, Moi Hoc Mon, Tan Binh and Pham Van Hai Markets as shown in Figure 1), 7 samples at vegetable fields and 8 ready-to-eat raw vegetables at restaurants. While collecting vegetables, thirty-three vegetable-related water samples were obtained. The water-sample set included 4 groundwater samples for irrigation, 10 samples at suburban irrigation canals (which were also used for washing vegetables), 5 samples for washing vegetables at the food markets, 7 samples from the drainage canals in the center of HCMC and 7 tap water samples at the markets and restaurants. Additionally, eleven river water samples were collected along Saigon River at 140 km of upstream from the river mouth (i.e., ca. 4 km downstream of Dau Tieng Reservoir). River waters were sampled twice a year, in dry and rainy seasons, namely, in March and September, respectively in 2005 and 2006. All samples were put into sterile plastic bags/bottles and kept in the ice boxes until the bacterial indicators were enumerated in the laboratory on the same day.

**Measurement procedures**

The surfaces of vegetable samples were wiped with a sterile glass wool to capture microorganisms, which were subsequently eluted by vigorously shaking the glass wool for more than 15 seconds in a 15 mL of 0.05 N glycine-0.14N NaCl solution (pH 7.5). The eluate was then diluted to appropriate concentration for the enumeration by filtering through an Analysis Monitor (0.45 μm pore size; Millipore) using the m-ColiBlue24 culture broth (Millipore). The weight of each vegetable was measured by a digital balance to...
calculate the number of bacteria per unit weight of the vegetable, i.e. CFU/g. This eluate was also used to indentify the human enteric viruses such as NV-G1, NV-G2, AdV, EV and HAV, and the virus recovery of this method varied from 6.8% to 68% depending on core or leaf samples (Kitajima et al. 2006). The wiping method was selected to make detachment of microbes from folder leaves more efficient, and because the glass wool wiping method was used to trap the viruses in many studies (Menut et al. 1993; Vivier et al. 2004; Lambertini et al. 2008). Meanwhile, no pre-treatment was done with water samples before measuring the bacterial indicators. After 24-hour incubation at 37°C, E. coli was counted as the number of the blue colonies, whereas TC was enumerated as the sum of the blue and red colonies.

RESULTS AND DISCUSSION

Bacterial contamination of raw vegetables

Figure 2 shows the numbers of E. coli and TC on raw vegetables collected at various sampling sites. Most of the tested vegetables were positive with bacterial indicators regardless of the sampling sites. As shown in Figure 2, vegetables harvested in the field had adsorbed lower counts of TC than vegetables obtained at restaurants and markets. In the case of vegetables from fields, the major sources of bacterial contamination may be attributable to the irrigation water and to the organic fertilizer containing animal feces, which is commonly used in Vietnam. Vietnamese farmers normally spray the composted animal feces directly to the soil near the crop’s root areas instead of spraying on the crop’s leaves. In our study, only the leaves of raw vegetables were tested and all vegetable samples were from the good agricultural practice (GAP) fields, therefore, the risk of bacterial contamination of the raw vegetables could be lower in these agricultural fields than other fields.

In the case of vegetables in food markets, 100% or 76% of samples were contaminated with TC or E. coli, respectively. Interestingly, vegetables sold in the markets and served in the restaurants showed a trend of higher bacterial contamination than those collected at the cultivation fields, suggesting the additional contamination during transportation and/or washing procedures after harvesting. The additional bacterial contamination may occur due to: (i) harvested vegetables could come into contact with other bacteria-contaminated sources during harvesting and transportation such as contaminated soil, irrigation water, containers or vegetables; (ii) harvested vegetables could be contaminated by contact with other bacteria-contaminated sources at the markets, including washing containers, washing water, infectious vegetables or unhygienic conditions; and/or (iii) bacteria may multiply on the surface of raw vegetable after harvesting (http://www.agf.gov.be.ca). Less E. coli and TC were found on the vegetables at restaurants and 37% of the samples were negative for E. coli, indicating that these bacteria could be removed in part by washing vegetables with clean water. However, even on the ready-to-eat vegetables, the quantity of E. coli or TC was quite high. TC counts varied from 2 log to 5 log/g, which is 100 times higher than the acceptable maximum number of TC in fresh vegetable, 3 log CFU/g; and 10,000 times higher than TC limitation of clean and safety vegetables recommended by the Vietnamese Ministry of Health. As for the two vegetable species, i.e. mint (Ocimum tenuiflorum L.) and salad (Lactuca sativa L.), salad had higher contamination rates of TC and E. coli than mint because salad has broader leaves and open leaf structure, which gives a higher chance of contact with bacteria.
Bacterial contamination of vegetable-related water and Saigon River

Figure 3 shows the bacterial indicators detected in the various water samples. It is notable that the suburban canal waters were contaminated with TC almost to the same levels as in the drainage canals in the city center. About 80% of suburban canal water was positive for *E. coli*. Only 20% of suburban canal water met the Vietnamese standard of TC for irrigation water, i.e. 100 CFU/mL, while ca. 40% of samples contained 10 times higher concentration than 100 CFU/mL. Although groundwaters used for irrigation were also contaminated with TC, only one groundwater sample contained *E. coli*. The groundwater and suburban canal waters were commonly used for irrigating or washing vegetable before shipping to the market. Because it was found that these waters are contaminated with bacteria, irrigating and washing vegetables could increase the risk of bacterial contamination on vegetables. While 100% and 60% of vegetable wash water at food markets was contaminated with TC and *E. coli*, no tap water at markets or restaurants showed bacterial contamination. During the vegetable sampling, we found that vegetables were often washed in the contaminated suburban canals to remove soil and dirt. This observation, along with the aforementioned experimental results, suggests that washing vegetables in contaminated canals by the wholesalers can be the primary reason for the increased bacterial counts in the markets.

Figure 4 shows the TC profiles in Saigon River. In the lower SG River, TC concentration was always high due to heavy contamination by the drainage from HCMC. In the most upstream section, i.e. 140 km, TC concentration went up in the rainy season (September) due to run off of animal wastes. In the middle section between 50 and 85 km, where most of vegetables are produced, water quality almost met the TC standard for irrigation and aquaculture purposes. At 55 km there is Ben Than Water Intake for water supply to HCMC, where the TC concentration was also higher than

Figure 4 | Total coliforms detected in Saigon River (The asterisks indicate no measurements and abbreviations of N indicate the negative results).
the Vietnamese Standard of TC as 5,000 MPN per 100 mL for river waters for drinking water supply (TCVN 5942-1995). In the lower section between 8 and 55 km, TC was always high due to high population density and industrial development.

**Risk analysis of bacterial contamination of raw vegetables**

Although fresh product is considered to have a lower risk compared to foods of animal origin, the risk of food-borne illnesses from fresh produce is real (Beuchat 1996). However, it is difficult to determine the exact source of contamination of human pathogens on fresh product/raw vegetables because at any point throughout production, harvesting, and handling, because the disease-causing microorganisms can come in contact with products by various means. In addition, the lack of data and information, e.g. the information on the relationship between the quantity of biological agents and the frequency and magnitude of adverse human health effects, makes accurate risk assessment more difficult.

Consequently, in the developing countries, health authorities want to know where intervention should be placed to reduce the health risks for consumers related to bacterial contamination. Figure 5 shows the spatial distribution of TC contamination of the vegetables and vegetable-related water samples obtained from the vegetable fields to wholesale markets and then delivery to retail markets. The results suggested that the bacterial contamination took place both pre- and post-harvest periods, however, the post-harvest contamination was found to be more significant. Contaminated irrigation water causes bacterial contamination of vegetables in the fields, but the number of TC found in vegetables at the retail markets increased significantly (Figure 5). The vegetable washing water at those markets was only slightly contaminated. Thus, it was estimated that the vegetables were

![Figure 5](https://iwaponline.com/wst/article-pdf/58/12/2403/436008/2403.pdf)
contaminated during the transportation from the agricultural fields to the wholesale markets or to the retail markets. Figure 6 shows the distribution of E. coli concentrations in the study area including irrigation canals in suburban areas (Cu Chi and Hoc Mon districts or upper HCMC), canals in HCMC center and the Saigon river along HCMC in September 2006 and their areal relationship. As shown in Figure 6, most canals were contaminated by E. coli. The city canals were polluted seriously, consequently, the central and lower sections of SG river were also affected. River
water at Ben Than water intake had high concentration of *E. coli* due to the impacts of the sewage drainages from Thu Dau Mot Town and of the upflow of contaminated water from HCMC due to tidal influences.

In addition, *E. coli* was also positive in 80% of water samples from the irrigation canals. If vegetable farmers or wholesalers use contaminated canal water for washing, vegetables will be easily contaminated and may even cause secondary contamination at markets. Saigon River was less contaminated with *E. coli* than canals but may work as a conveyor to transport microorganisms from severely polluted canals downstream to cleaner canals upstream due to the considerable tidal effect. Normally, most wholesalers wash the root vegetable in the irrigation canals to remove the soil and refresh vegetables before transporting them to the retail markets. At the markets, the sellers wash different vegetables in the same water bowl before selling them. The water and containers can easily contaminate relatively cleaner vegetables and cause a serious health risk to those who eat the uncooked vegetables.

**Control of food-borne pathogens in pre- and post harvest environments**

In general, the strategy for the prevention of microbial contamination of foods include: (i) preventing contamination; (ii) minimizing contamination; (iii) minimizing contact with human or animal feces; (iv) ensuring a safe water supply; (v) using manure and municipal biosolids safely; (vi) focusing on worker health and hygiene; (vii) following the laws and regulations and (viii) being accountable at all levels of agricultural environment (farm, packing facility, distribution center and transport operation). The program of “clean and safety vegetable production from farm to fork” should be launched to extend the existing agricultural extension program of “clean and safety vegetable production” (CSVP). The CSVP program is funded by HCMC People Committee and DARD, and is to be implemented between 2000 and 2010 covering 5,700 ha of the vegetable cultivation area. However, this program may not be sufficient if just to address only farming practices or Good Agricultural Practices (GAP); effort and attentions should also be devoted to improve the post-harvest environment and the surrounding water quality in order to eliminate health risks of pathogenic microorganisms associated with fresh vegetables.

In order to achieve the GAP more efficiently, the farmers need to protect the water sources from microbial contamination. The wholesaler who buy vegetables from the farmers should use clean, bacteria-free water for washing vegetables, and transport them to the markets or consumers as quickly as possible. At restaurants and households, they should wash raw vegetable carefully with potable water or disinfectant-containing water before eating raw vegetables. Washing the vegetables three times with tap water only or with the ozonated water can reduce the parasite infection rate only by 51.9% and by 50%, respectively (Hong 2007). The 200 ppm solution of chlorine reduces microbial population to one to ten percent. Trisodium phosphate and organic acid have high potential as disinfectants for vegetables (Beuchat 1998).

**CONCLUSIONS**

Raw vegetables in the market were found to be more contaminated with TC and *E. coli* than the vegetables in the agricultural field. Using microbiologically contaminated water for irrigation and post-harvest washing of vegetables was found to cause serious health risks in such regions as HCMC, where people have a tradition of eating raw vegetables. To reduce the health risks associated with the consumption of raw vegetables, it is evident from the study that the post-harvest handling practice should be first to be improved for safer agricultural practices. Providing enough clean water to wash vegetables, as well as reducing the microbial contamination of canals and rivers, can significantly reduce the risk of bacteria-causing illness amongst the people in HCMC. For this purpose, improving washing habits and raising the awareness of the hygienic conditions of raw vegetables and the awareness of microbial health risks of eating raw vegetables are also important tasks for the better health in the Vietnam and other developing countries.

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