Microbiological Quality of Fresh Produce in Southeast Asia: An Assessment of Risks, Challenges, and Opportunities

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ABSTRACT

The consumption and production of fresh produce have been steadily increasing. In Southeast Asia, increased production and consumption of fresh produce can be attributed to efforts to attain food security, promote export industries, and meet escalating consumer demands. However, the fresh produce industry in Southeast Asia faces many challenges that hinder its ability to provide safe and wholesome products. One of these issues is the microbial contamination of fresh produce, which is now widely acknowledged as a major contributor to food poisoning and other foodborne illnesses. Although the information on the microbiological quality of fresh produce is available in other countries, data are limited in most Southeast Asian countries. This paper discusses the microbiological quality of fresh produce in Southeast Asia and the major sources of microbial contamination of fresh produce in the context of certain agricultural practices in this region. This paper argues that the lack of information on the microbiological quality of fresh produce significantly undermines efforts to establish guidelines and standards for fresh produce quality and hinders understanding of the potential risks and impacts of fresh produce contamination. This paper concludes with a discussion of future prospects and intervention opportunities to address the problem of microbial contamination of fresh produce in Southeast Asia.

INTRODUCTION

Southeast Asia is a geographic subregion of Asia consisting of 11 countries divided into two areas: continental or mainland Southeast Asia comprising Vietnam, Laos, Cambodia, Thailand, Myanmar, and western Malaysia, and archipelagic or maritime Southeast Asia comprising Indonesia, eastern Malaysia, Singapore, East Timor, Brunei, and the Philippines. These countries are home to 630 million with a land area of a total of 4.5 million square kilometers (OECD-FAO 2017). The climate of each country varies; nevertheless, a major portion of the region has a tropical subhumid climate with a major form of vegetation in tropical rainforests. Some areas such as eastern Malaysia and eastern Philippines receive abundant rainfall throughout the year, whereas some areas such as Thailand and

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coastal Myanmar have a definite dry season but sufficient rainfall (Capistrano and Marten 1986).

Agriculture is one of the key sectors that drive the growth and development of Southeast Asian economies. It accounts for a significant portion of the region's gross domestic product, which is currently valued at US$ 2.6 trillion, and provides food, income, and livelihood to a substantial fraction of the population in the region (OECD-FAO 2017). Furthermore, agriculture in Southeast Asia plays an increasingly important role in the world agricultural food trade. Agricultural exports in the region, worth US$ 139 billion, significantly outweigh imports that were valued at US$ 90 billion in 2014 (OECD-FAO 2017).

Fresh produce is an agricultural commodity that is experiencing astronomical growth in production and consumption in Southeast Asia. Fresh produce is a broad term that covers a wide range of agricultural crops and goods, including fruits, vegetables, meat, and crops. More recently, fresh produce has come to be more commonly associated with fruits and vegetables that are kept fresh as when they were harvested. In this paper, the term "fresh produce" will be limited only to vegetables, one of the commodities that have the biggest increase in production and consumption in Southeast Asia.

The fresh produce scenario in Southeast Asia

Vegetable production in Southeast Asia has increased consistently over the past three decades (Ali 2000; Johnson et al. 2008). The total volume of vegetable production in the 1990s was 10.7 metric tons, and it increased to 13.9 metric tons in 2000 (Shukor et al. 2001). During the 2000s, approximately 2.4 million hectares of agricultural land were utilized to produce 22.5 million tons of vegetables (Ali 2000). In 2012, total vegetable production reached 34.2 million tons, of which 1.3 million tons were exported globally. Furthermore, 2.4 million tons of vegetables were imported into the region, for a total of 32.6 million tons of vegetables available for consumption (Hughes et al. 2015). Chili pepper, tomato, yardlong bean, common cabbage, and shallot are among the important vegetables harvested in this region. The volume of various types of vegetables produced in each country varies according to the relative importance and local demand for individual vegetables. This provides an ideal opportunity for each country to share its experiences and expertise for the successful cultivation of its major vegetables (Ali 2000).

The growing agricultural production of fresh produce in Southeast Asia can be attributed to the widespread perception among consumers that fresh produce is an essential component of a balanced diet and healthy lifestyle. Indeed, the consumption of fresh produce provides a variety of nutrients such as potassium, vitamin C, fiber, and other phytochemicals, and has been linked to a reduction in the risk of numerous chronic diseases, such as diabetes, heart disease, and certain cancers (Jung et al. 2014). Although this consumer perception has been observed in several Southeast Asian cities (Vital et al. 2014), the increase in agricultural production in the region is primarily the result of efforts to achieve food security and promote export industries (Johnson et al. 2008), particularly as the global demand for fresh produce has skyrocketed in recent years. Other contributors to this growth include urbanization, growing incomes, physical infrastructure development, and the increase in the level of technology applied for agricultural production (Ali 2000; Everaarts and de Putter 2009).

Microbiological quality of fresh produce in Southeast Asia

The ongoing expansion of the fresh produce market in Southeast Asia is not without obstacles. In reality, the fresh produce industry faces challenges that impede its capacity to provide safe, wholesome, and sufficient vegetables to meet growing consumer demand. Climate change and extreme climate events, increased urbanization that puts pressure on arable land, an aging agricultural population, pests and infestations, microbial contamination, and unfavorable policies toward agriculture are some of these hurdles (Everaarts and de Putter 2009; Hughes et al. 2015).

In Southeast Asia, microbial contamination of fresh produce is an underrated but significant issue. Although several developed countries have reported and acknowledged microbial contamination of fresh produce as an imminent threat to food safety (Lynch et al. 2009; Callejón et al. 2015; Uyttendaele et al. 2015), data on the microbiological quality of fresh produce are only available in a small number of Southeast Asian countries (Vital et al. 2014). In fact, only six countries including Vietnam, Thailand, Myanmar, Malaysia, Singapore, and the Philippines have reported on the microbiological quality of fresh produce. With its role as one of the top producers of vegetables in the world (OECD-FAO 2017), the fresh produce microbiological quality in Southeast Asia must be evaluated and monitored to create baseline information regarding its level of microbial contamination in the region and to develop microbial risk assessment models geared toward fresh produce safety.

Microorganisms that have been reported in fresh produce are varied, including bacteria, protozoa, fungi, and helminths (Table 1). For instance, a microbial assessment of 12 types of vegetables, considered essential parts of the Vietnamese diet and usually consumed raw, in Hue City, Vietnam, showed that these commodities are highly contaminated with different microorganisms including bacteria such as *Escherichia coli* and *Salmonella* spp.; protozoa such as *Cryptosporidium* spp., *Isospora* spp., and *Cyclospora* spp.; and helminths such as *Fasciola* spp., *Ascaris* spp., *Trichuris* spp., and *Clonorchis sinensis* (Chau et al. 2014). In a similar study by Ha and Pham (2006) and Van Ha et al. (2008), lettuce, and mint surveyed in two cities in Vietnam, namely, Ho Chi Minh, and Hanoi, were found to be contaminated with coliform bacteria and *E. coli*. Meanwhile, ready-to-eat vegetables in hospitals in Thailand were shown to be contaminated with fecal coliforms, *E. coli*, *Salmonella* spp., *Vibrio* spp., and *Aeromonas* spp. (Dhiraputra et al. 2005).

The study of Srisamran et al. (2022) focused on the finding and analysis of the indicator and pathogenic bacteria found in fruits and vegetables retailed in markets and found that there are higher concentrations of fecal coliforms and *E. coli* in open markets in comparison to supermarkets. Moreover, the prevalence of *Salmonella* and *Shigella* was less than 5%, and no positives were found for *Listeria monocytogenes.* It was also found that *Salmonella* contamination is higher during the rainy season as compared to the dry season (Srisamran et al. 2022).

In an informal vegetable market in Cambodia, Desiree et al. (2021) investigated the *Salmonella* sp. and fecal indicator organisms, *E. coli* and coliforms, in loose-leaf lettuce, tomato, and cucumber samples from different vendors and markets. It was reported that *S. enterica* was the highest in lettuce among the produce, regardless of the season. For *E. coli*, the highest prevalence was found in lettuce and higher occurrence during the rainy season when compared to during the dry season. The coliform amounts of lettuce and tomatoes were highest in the rainy season. The study indicated seasonal patterns of microbial prevalence and high bacterial contamination in the informal vegetable market in Cambodia (Desiree et al. 2021).

From 2017 to 2019, Nguyen et al. (2021) collected fresh vegetables to study the contamination of fresh vegetables in Vietnam. It was reported that the *Salmonella* isolation rate was higher in the rainy season than in the dry season. Of the isolated

Salmonella, Weltevreden was reported to be the most prominent serovar in fresh vegetable in wet markets. Additionally, all *S.* Weltevreden isolates were susceptible to different antibiotics examined. Fresh vegetables are therefore seen to be a possible means of transmitting *Salmonella* to the people in the Mekong Delta of Vietnam (Nguyen et al. 2021).

In Singapore, samples of vegetables commercially available in supermarkets and local markets were surveyed for microbiological quality and were found to be contaminated by aerobic and coliform bacteria. Interestingly, no pathogenic microorganisms have been recovered from the samples (Seow et al. 2012). This is discordant with the result of Kuan et al. (2017) in Malaysia where pathogenic bacteria such as Shiga toxin-producing *E. coli* non-O157:H7, *Salmonella* spp., and *L. monocytogenes* were observed, besides aerobic and coliform bacteria, and molds, and yeasts in vegetables cultivated using conventional and organic farming. Similarly, the vegetables in West Java, Indonesia, harbor pathogenic microorganisms such as *Bacillus cereus*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* besides *E. coli*, and coliforms (Hassan and Purwani 2016).

Comprehensive assessments in the Philippines have likewise revealed microbial contamination of fresh produce. A study by Vital et al. (2014) and Vital et al. (2017) showed the pervasiveness of pathogenic *Salmonella* spp., thermotolerant *E. coli*, and somatic coliphages in fresh produce that are typically consumed raw and minimally processed in supermarkets and wet markets in major urban centers in Luzon, Philippines. Additionally, the same microorganisms were recovered in fresh produce surveyed in urban farms in Metro Manila (Garcia et al. 2015). Collectively, this indicates that there is microbial contamination in fresh produce in the Philippines at the production and retail levels.

Different fresh produce of different varieties has been variably studied in Southeast Asian countries (Table 1). For example, data on the microbiological quality of cabbage, lettuce, and tomato are available for most countries, whereas information on the quality of other varieties such as cilantro, mint, and parsley have been limited to only a few countries. This may be probably because of the differences in demand and relative importance of the diverse types of fresh produce in each country. Nonetheless, a similar subset of microorganisms that includes *E. coli* and *Salmonella* spp. have been uniformly recovered from various fresh produce of different types in different countries.

Fresh produce contamination in Southeast Asia has also renewed public interest in its crucial role in outbreaks of foodborne diseases. The World Health Organization (2016) reported 150 million episodes of foodborne illnesses resulting in 175,000 deaths and an economic burden of 12 million disability-adjusted life years in Southeast Asia. Although contaminated fresh produce has been identified as a vehicle for foodborne diseases and is widely implicated in outbreaks in developed countries with stringent food safety practices, notably, no well-studied outbreaks of foodborne diseases associated with fresh produce have been reported in Southeast Asia. This may be attributable to the lack of knowledge regarding the level of microbial contamination in fresh produce in Southeast Asia, as well as lack of efficient surveillance and traceability systems, which make it difficult to establish a connection between fresh produce pathogens and foodborne illnesses and to trace their origin.

Table 1: Microbiological quality of fresh produce in some Southeast Asian countries.

Vegetable (scientific name)	Country	Microorganisms detected	Reference
basil (*Ocimum basilicum*)	Vietnam	*E. coli*	Chau et al. 2014
	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
bean sprout (*Vigna radiata*)	Philippines	*E. coli*	Vital et al. 2017
	Singapore	*Salmonella*	Zwe and Yuk 2017
bell pepper (*Capsicum annuum*)	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
	Philippines	*E. coli* *Salmonella*	Vital et al. 2014 Vital et al. 2017
cabbage (*Brassica oleracea*)	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
	Philippines	*E. coli* *Salmonella*	Vital et al. 2014 Garcia et al. 2015
carrot (*Daucus carota*)	Malaysia	*Listeria* *Salmonella*	Kuan et al. 2017
	Philippines	*E. coli* *Salmonella*	Vital et al. 2014 Vital et al. 2017
celery (*Apium graveolens*)	Vietnam	*E. coli* *Salmonella*	Chau et al. 2014
	Thailand	*Aeromonas* *E. coli* *Vibrio*	Dhiraputra et al. 2005
cilantro (*Coriandrum sativum*)	Vietnam	*E. coli*	Chau et al. 2014
cucumber (*Cucumis sativus*)	Malaysia	*Listeria*	Kuan et al. 2017

		Salmonella	
lettuce (*Lactuca sativa*)	Vietnam	*E. coli* *Salmonella*	Van Ha et al. 2008 Chau et al. 2014
	Thailand	*Aeromonas* *E. coli* *Vibrio*	Dhiraputra et al. 2005
	Malaysia	*Listeria* Shiga toxin-producing *E. coli* non-O157:H7	Kuan et al. 2017
	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
	Philippines	*E. coli* *Salmonella*	Vital et al. 2014 Vital et al. 2017
mint (*Mentha*)	Vietnam	*E. coli*	Van Ha et al. 2008
parsley (*Petroselinum crispum*)	Thailand	*E. coli*	Dhiraputra et al. 2005
spinach (*Spinacia oleracea*)	Vietnam	*E. coli* *Salmonella*	Chau et al. 2014
	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
	Philippines	*E. coli* *Salmonella*	Garcia et al. 2015
tomato (*Solanum lycopersicon*)	Thailand	*Aeromonas* *E. coli*	Dhiraputra et al. 2005
	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016
	Philippines	*E. coli* *Salmonella*	Vital et al. 2014 Vital et al. 2017
watercress (*Nasturtium officinale*)	Vietnam	*E. coli* *Salmonella*	Van Ha et al. 2008
zucchini (*Cucurbita pepo*)	Indonesia	*B. cereus* *E. coli* *Salmonella* *Shigella* *S. aureus*	Hassan and Purwani 2016

Some microorganisms of interest in fresh produce in Southeast Asia

Escherichia coli

E. coli, described as a Gram-negative, facultatively anaerobic, rod-shaped bacterium, is one of the most prevalent microorganisms in fresh produce and other agricultural samples. It is usually found in the gastrointestinal tract and has been associated with diarrheal diseases (Tenaillon et al. 2010; Njage and Buys 2015). Furthermore, it is a popular indicator microorganism used to monitor pollution of different resources as its presence relates to fecal contamination (Carlos et al. 2010; Odonkor and Ampofo 2013; Widmer et al. 2013; Uyttendaele et al. 2015). Although the presence of *E. coli* does not always relate to the presence of pathogenic strains such as O157:H7, it is a conservative surrogate for a variety of microorganisms in different resources in an agricultural setup (Allende and Monaghan 2015).

A pathogenic strain of *E. coli, E. coli* O157:H7, has been associated with numerous foodborne disease outbreaks related to fresh produce (Harris et al. 2003; Lynch et al. 2009; Callejón et al. 2015). In 2006, an outbreak in different states of gastrointestinal infections linked with the ingestion of O157:H7-contaminated spinach was reported in the United States (Wendel et al. 2009). There were 255 confirmed cases that resulted in five fatalities and 116 hospitalizations (Sharapov et al. 2016). Multiple factors have contributed to the success of O157:H7 to survive in various environments, including fresh produce. For example, its remarkable adaptability allows it to survive adverse conditions, such as nutrient fluctuations (Chekabab et al. 2013).

Salmonella spp.

Salmonella spp. are Gram-negative, rod-shaped bacteria that are the leading causes of foodborne diseases in the United States that account for an estimated 1.4 million cases and a cost of US$ 2.5 billion in medical expenditures and lost productivity (Hanning et al. 2009). The presence of *Salmonella* spp. has been reported in different food products. Furthermore, they are the leading cause of outbreaks caused by fresh produce contamination, accounting for 46% of foodborne disease outbreaks in the United States (Hanning et al. 2009; Callejón et al. 2015). In the Philippines, the Department of Health reported 13,117 cases and 58 deaths in 2022 (Epidemic-Prone Disease Case Surveillance DOH, 2023). The persistence of *Salmonella* spp. on fresh produce may be attributed to their ability to grow in high densities and survive extensive conditions during transportation and prolonged storage associated with vegetable production (Lynch et al. 2009).

Listeria monocytogenes

Listeria spp. are a group of bacteria that are widely distributed in different environmental samples. One member of this group, *L. monocytogenes*, causes many illnesses of varying severity, from mild gastroenteritis to infection of the nervous system (Zhu et al. 2017). Alarmingly, *L. monocytogenes* is increasingly being recovered in different food products such as fresh produce resulting in an increased incidence of outbreaks in recent years. Generally, there is a decreased incidence of listeriosis outbreaks associated with meat and dairy products in the United States; however, listeriosis caused by contaminated fresh produce and dairy products showed no decrease in frequency (Buchanan et al. 2017).

Bacillus cereus

B. cereus is a motile, Gram-positive, facultatively anaerobic bacterium that is found usually in natural environments such as soil, water, and plants as well as in food products where it is normally associated with food poisoning. It causes foodborne illnesses, namely, emetic and diarrheal syndrome (Bottone 2010; Kim et al. 2016). *B. cereus* is an emerging major pathogen in fresh produce that has a contamination rate of 37.5% (Kim et al. 2016). A total of 103 outbreaks related to *B. cereus* have been described as of 2008, representing 1%–2% of the total outbreaks in the United States and 4%–5% of outbreaks in France (Tewari and Abdullah 2015).

***Shigella* spp.**

Shigella spp. are a group of Gram-negative, facultatively anaerobic, rod-shaped bacteria that are not frequently recognized as a cause of foodborne disease outbreaks (Reller et al. 2006). In recent years, however, it is increasingly being recovered in food products such as fresh produce resulting in increased risks of foodborne infections. For instance, several restaurant outbreaks of shigellosis caused by *Shigella flexneri*, epidemiologically linked to tomatoes, happened in the United States in 2001 (Reller et al. 2006). Likewise, an outbreak of shigellosis traced to a commercially available bean dip has been reported in 406 persons in the United States in 2000 (Kimura et al. 2004).

Potential sources of microbial contamination of fresh produce

Microbial contamination of fresh produce can occur at any point along the "farm-to-fork" continuum, which includes the entire production chain from agricultural farms, processing facilities, transport, and distribution, retail establishments, and households. Moreover, the microbiological contamination of fresh produce may be affected by various production-related factors, such as sanitation, hygiene, the quality and delivery of irrigation waters, the duration between irrigation and harvest, weather and storage conditions, and delivery to markets (Pachepsky et al. 2011).

Vegetables are typically susceptible to microbial contamination due to the complex interaction of these factors and their natural properties. Due to how they are handled, transported, prepared, or consumed, several types of fresh produce are more prone to microbial contamination. These include leafy vegetables and herbs including cabbages, chicory, lettuce, spinach, watercress, and leafy herbs such as basil, cilantro, and parsley (FAO-WHO 2008). These varieties, also known as "leafy greens," are typically processed minimally or consumed raw, especially in many Southeast Asian cuisines and cultures, as ingredients to salad preparations or as condiments (Chau et al. 2014; Ravaliya et al. 2014; Vital et al. 2014). Additionally, these varieties do not typically undergo microbial inactivation or preservation treatments as these processes may adversely impact the sensorial qualities of fresh produce resulting in browning, discoloration, loss of taste, texture, and nutrients (FAO-WHO 2008; Gil et al. 2009). Furthermore, partial interventions such as washing with chemical sanitizers do not completely remove the presence of microorganisms as they may be localized not only in external apertures of fresh produce such as stomata and roots but within plant tissues, which are not typically reached by the limited penetration of sanitizers (Solomon et al. 2002; Gil et al. 2009; Erickson 2012; Wright et al. 2013; Jung et al. 2014). Collectively, this poses a significant risk to the health and safety of consumers as they may be exposed to potentially viable and pathogenic microorganisms.

Numerous etiologic sources of microbial contamination in fresh produce have been identified and have been loosely categorized as preharvest and postharvest sources. Preharvest factors include current technologies for the production of raw agricultural materials, whereas postharvest sources include technologies of storage, transportation, and processing of agricultural raw materials into food products (Wos 1985).

Fecal material

A recognized preharvest and postharvest source of fresh produce microbial contamination is wildlife, livestock, and human fecal material. In fact, not only the density of wildlife and livestock is considered a critical factor in the damage of crops, but it also increases the transmission of microorganisms and fecal contamination of fresh produce. As animals roam in agricultural farms, they may shed, and drop feces harboring pathogenic microorganisms, namely, *E. coli* O157:H7, *Salmonella* spp., *Listeria* spp., and *Campylobacter* spp., which may directly contaminate fresh produce cultivated in farms. Indirect fecal contamination may also occur when the production environment where fresh produce is cultivated, and surface waters used for irrigation becomes contaminated by fecal material (Cooley et al. 2007; FAO-WHO 2008; Gil et al. 2009). A common practice in large farms in Southeast Asia is confining animals within livestock facilities to separate them from the field and to prevent crop damage. However, runoff from these facilities may also contaminate soil and water used in the cultivation of fresh produce.

Fresh produce may also be contaminated by fecal material through the deliberate use and application of raw and composted manure, excreta, and sewage sludge to soils as amendments or fertilizers (Cooley et al. 2007; FAO-WHO 2008; Gil et al. 2009). Agricultural soils in certain parts of Southeast Asia have been exhausted of nutrients because of having been farmed for successive generations. Consequently, the use of low-cost and highly available organic wastes such as fecal material has been practiced to supply nutrients and increase the fertility of agricultural soils. Nevertheless, the use of this resource has adverse impacts on the microbiological quality of fresh produce as they are reservoirs of potentially pathogenic microorganisms (Lam et al. 2015).

In Southeast Asia, nowhere is the use of fecal material for fertilizer more commonly practiced than in Vietnam where its government prohibits the use of excreta for agriculture (Jensen et al. 2008). Excreta use for crop agriculture in Vietnam was estimated to represent US$ 83 million in fertilizer import savings. Furthermore, it has yielded moderate savings for individual farmers representing a sizable fraction of their annual income (Tran-Thi et al. 2017). Although excreta is a recognized vehicle of fresh produce contamination in Vietnam, the possibility of replacing it with inorganic fertilizers is very unlikely, especially as the cost of industrially produced inorganic fertilizers continues to increase (Phuc et al. 2006).

Irrigation water

Irrigation waters are also critical preharvest and postharvest sources of microbial contamination on fresh produce. In most agricultural settings in Southeast Asia, irrigation waters are derived from sources ranging from municipal water, rainwater, groundwater, surface water, canals, and seeps. Although certain sources such as municipal water and groundwater are suitable for irrigation, they are quite expensive and have limited availability (Uyttendaele et al. 2015). This has led to the rampant and widespread use of openly available yet highly polluted sources such as surface waters, canals, and seeps for irrigation although they are usually contaminated by agrarian runoffs, fecal material coming from livestock and wildlife, wastewater discharge, and septic leakage (Steele and Odumeru 2004; Lynch et al. 2009; Pachepsky et al. 2011; Jung et al. 2014; Allende and Monaghan 2015; Garcia et al. 2015; Uyttendaele et al. 2015). This is particularly true in many low-income and poor countries of Southeast Asia where there is a limited quantity of water that can be used for irrigation (Garcia et al. 2015).

Contaminated waters have been found to harbor pathogenic microorganisms including bacteria such as *B. cereus*, *Campylobacter* spp., *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., *S. aureus*, and *Yersinia enterocolitica*; protozoa such as *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia* spp., and *Entamoeba histolytica*; and viruses such as adenoviruses, enteroviruses, noroviruses, and rotavirus (Lynch et al. 2009; Pachepsky et al. 2011). These pathogens can be passed on to fresh produce directly when contaminated water is used in the irrigation of soil, application of pesticides, and postharvest processing of farm products (Harris et al. 2003; Jung et al. 2014; Uyttendaele et al. 2015).

In a study by Widmer et al. (2013), surface waters in four main rivers used for agricultural irrigation in major Southeast Asian cities were reported to have been contaminated with *E. coli*. These include the Citarum River in Indonesia, the lower Chao Phraya in Thailand, the Saigon River in Vietnam, and the Tonle Sap-Bassac in Cambodia. Alarmingly, a small proportion of *E. coli* recovered from these surface waters was pathogenic as demonstrated by the presence of virulence genes. The same results were obtained by Vital et al. (2017) where surface water samples from rural, suburban canals, and urban runoff canals in Ho Chi Minh City (Vietnam) contained varying levels of *E. coli*. Surface waters utilized for irrigation in the Philippines likewise showed similar outcomes. In the study of Garcia et al. (2015), irrigation waters sourced from the Marikina River in Metro Manila were contaminated by *E. coli* and *Salmonella* spp. This indicates that the use of surface waters in major Southeast Asian rivers for irrigation may potentially compromise the quality of agricultural produce.

Human activity

People involved in the primary production of fresh produce are important to point source of microbial contamination of fresh produce. These include growers, packers, shippers, distributors, and handlers that may harbor microorganisms in their hands, whether bare, or gloved, that may inadvertently cross-contaminate fresh produce (Suslow et al. 2003; Gil et al. 2009; Jung et al. 2014). Fresh produce may become contaminated during harvest by contact with contaminated harvesting tools including lawnmowers, conveyor belts, knives, and containers like bins, crates, and buckets. Furthermore, additional practices such as trimming and coring of lettuce increase human handling and microbial infiltration through cuts which allow opportunities for contamination (Gil et al. 2009; Jung et al. 2014).

Meanwhile, processing practices such as grating or cutting, washing, or sanitizing, packing, and storing likewise represent alternative routes to fresh produce contamination. For instance, grating and cutting of fresh produce release nutrient-rich exudates that may facilitate microbial growth and create cuts where microorganisms can infiltrate. During washing and sanitizing, the waters used may harbor high amounts of pathogenic microorganisms which may possibly contaminate the fresh produce. Although the use of disinfectants and sanitizers such as chlorine wash removes surface microorganisms, soil particles, and unwanted debris, they have limited utility as microorganisms may infiltrate deeply into the natural apertures of fresh produce such as stomata and artificial cuts, cracks, and crevices and within internal tissues (Solomon et al. 2002; Gil et al. 2009; Erickson 2012; Jung et al. 2014).

Antimicrobial resistance among microorganisms in fresh produce

The increase of antimicrobial resistance (AMR) among bacteria has made the issue of microbial contamination of fresh produce in Southeast Asia worse. AMR can be defined as the ability of bacteria to survive in the presence of an antimicrobial, when an antimicrobial is used according to a certain regimen, as a consequence of genetic changes. (Drlica and Perlin 2011; Corona and Martinez 2013). The rise of antibiotic-resistant bacteria (ARB) and spread of antibiotic-resistant genes (ARG) are primarily driven by the widespread and increasing use of antimicrobials in different environments including clinical, veterinary, and agricultural settings. These factors end up contaminating the fresh produce (Figure 1).

In Southeast Asia, the emergence and dissemination of AMR is a recognized problem, and several global hotspots of AMR have been identified in the region. These include the Red River Delta in Vietnam and the northern suburbs of Bangkok in Thailand (Van Boeckel et al. 2015), areas that play a critical role in agriculture in each country. However, reports on the AMR of fresh produce bacteria are few in Southeast Asia. In a study by Vital et al. (2017), *E. coli* and *Salmonella* spp. recovered from fresh produce in open-air markets and supermarkets in Luzon, Philippines, exhibited monoresistance, and multiresistance to commonly used antibiotics such as tetracycline, chloramphenicol, ciprofloxacin, and nalidixic acid. Additionally, genes conferring resistance to tetracycline and chloramphenicol were detected in most isolates.

Meanwhile, fresh produce retailed in Thailand was found to be contaminated with *S. enterica* that are resistant to tetracycline and streptomycin, and *Listeria* spp. that are resistant to tetracycline, penicillin, and chloramphenicol (Stonsaovapak and Boonyaratanakornit 2010; Lertworapreecha et al. 2013). The production of extended-spectrum β-lactamase, which confers resistance to β-lactam antimicrobials, was also detected in *E. coli* in fresh produce in Bangkok, Thailand (Boonyasiri et al. 2014).

In Malaysia, *L. monocytogenes* resistant to penicillin, meropenem, and rifampicin, and *Salmonella* Enteritidis exhibiting resistance to nalidixic acid, trimethoprim–sulfamethoxazole, and chloramphenicol were recovered from fresh produce in supermarkets (Kuan et al. 2017). Moreover, Thung et al. (2020) analyzed the resistance profile of *Salmonella* strains in different antimicrobial agents in an organic farm and tested brinjal, ladies' fingers, cucumbers, and soil samples. The study reported that all samples were resistant to penicillin and vancomycin. (Thung et al. 2020).

A different strain of *Salmonella*, Weltervreden, from fresh produce in Bogor, Indonesia, was found to be resistant to erythromycin, streptomycin, and sulfamethoxazole (Boonyasiri et al. 2014). Meanwhile, bell peppers (*C. annuum*) retailed in Vietnam were found to be contaminated with cefotaxime resistant *Klebsiella pneumoniae* (Harada et al. 2017).

The direct causality of ARB in fresh produce is difficult to ascertain as the vegetable supply chain in Southeast Asia is complex. However, studies have shown a close association between the prevalence of ARB and the use of antimicrobials in agriculture (Van Boeckel et al. 2015). Particularly, agricultural environments have been an important reservoir of AMR because of the rampant use of antimicrobials in intensive livestock and aquaculture operations in Southeast Asia (Nhung et al. 2016; Holloway et al. 2017). For many decades, antimicrobials traditionally used in human medicine have been extensively and indiscriminately used to prevent, contain, and treat diseases and enhance the growth of agricultural animals, including livestock, poultry, and fish (Kim and Ahn 2022). This imposes a selective evolutionary pressure that leads to the emergence of AMR among bacteria of animal origin (Van Boeckel et al. 2015). Furthermore, antimicrobials that are not absorbed by the body become residues that are eventually released in agricultural environments, along with ARB, through animal excretion (Zhu et al. 2013).

Consequently, animal wastes harboring antimicrobial residues and ARB may be introduced to fresh produce through their deliberate use as fertilizers and through contamination of soils where fresh produce is cultivated (Cooley et al. 2007; FAO-WHO 2008; Gil et al., 2009; Garcia et al. 2015). Moreover, animal wastes may be introduced to aquatic milieus such as surface waters that are considered to be ideal environments for the emergence and dissemination of AMR. In aquatic environments, antimicrobial residues are usually degraded and diluted resulting in trace and subinhibitory concentrations that may serve as regulatory molecules and substances in bacteria. Low levels of antimicrobials in aquatic environments create a "mutant selection window" that potentially selects and preserves ARB. Furthermore, ARB may disseminate AMR in aquatic environments through horizontal gene transfer of mobile genetic elements such as transposons and integrons (Suzuki et al. 2013).

The usage of contaminated surface waters for irrigation in Southeast Asia constitutes a potential health hazard as it may facilitate the transfer of ARB to fresh produce. Studies reported that the surface waters that are used for irrigation in some Southeast Asian cities harbor ARB and antimicrobial residues. For instance, Suzuki et al. (2013) found that surface waters in Metro Manila, Philippines, and Vietnam were contaminated by remains of sulfonamides, trimethoprim, and lincomycin. Furthermore, genes, namely, *sul1*, *sul2*, and *sul3*, conferring resistance to sulfonamide were detected. Meanwhile, antimicrobial-resistant *E. coli*-bearing integrons that are perceived to be the major source of AMR were recovered from irrigation waters in major localities in an agricultural province in the Philippines (Paraoan et al. 2017).

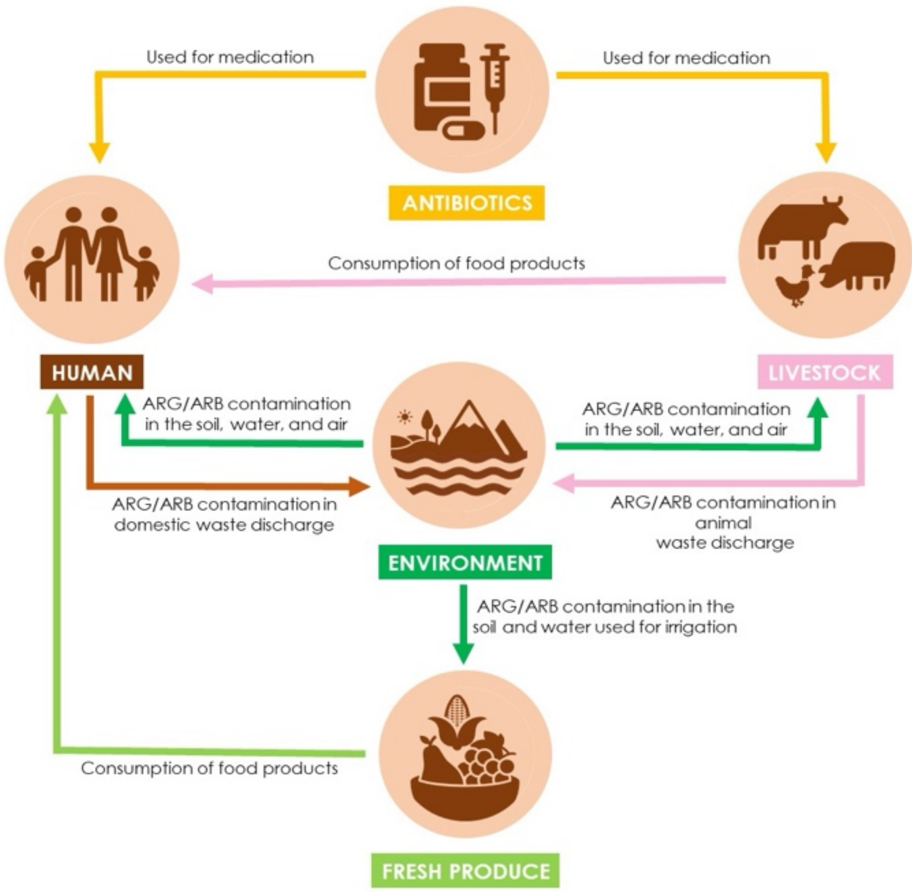

Figure 1: Transmission dynamics of antibiotic-resistant genes and bacteria among humans, livestock, environment, and fresh produce.

Future prospects and opportunities

Research on microbiological quality and microbial contamination of fresh produce

The lack of comprehensive information about the microbiological contamination of fresh produce in Southeast Asia remains to be the overriding factor that impedes a full understanding of the risks and challenges it entails. This includes the general lack of baseline data on the type of microorganisms present in fresh produce, including their quantity, prevalence, and their pathogenicity. Such information is crucial in any attempt to establish safety guidelines and microbial limits associated with vegetable production that may serve as regional standards and may guide farmers, consumers, and policymakers in preventing fresh produce contamination. This emphasizes the need for more research and studies on the microbiological quality and contamination of fresh produce in Southeast Asian countries to arrive at comprehensive food safety standards and proactive food safety strategies that may be applied and followed by the countries in the region.

Likewise, the lack of information on foodborne illnesses associated with fresh produce contamination in Southeast Asia undermines and blurs attempts to accurately estimate the actual risks and negative impacts microbial contamination of fresh produce has on the economy, public health, and society. This calls for active monitoring and surveillance systems to track cases of fresh produce-associated outbreaks in Southeast Asia and collect epidemiological data for rapid identification, prevention, and mitigation of future outbreaks.

Enhanced research is also needed to evaluate the fresh produce supply chain in Southeast Asia for the identification of potential sources of microbial contamination. These include scientific source tracking of major microbial contaminants for purposes of traceability.

Introduction and strict implementation of good agricultural practices

The complexity of the processes involved in the vegetable supply chain in Southeast Asia from cultivation and postharvest processing of vegetables in farms, to distribution and transport to markets, and eventually to food preparations in households significantly contribute to microbial contamination of fresh produce. Although these processes typically allow opportunities for contact between microorganisms and fresh produce, these may also serve as opportunities for preventive interventions. Good agricultural practices (GAPs) are a set of preventive methods ensuring that on-farm practices ensue in safe and wholesome agricultural produce as it reaches the farm gate. The application of these practices on the farm is needed to ensure food safety throughout the preproduction, production, harvest, through postharvest stages (FAO 2016).

The microbial contamination of fresh produce in Southeast Asia makes GAP enormously important for countries in the region. Although GAP schemes have been well-established in many developed countries, they are yet to be realized in many Southeast Asian countries. In fact, only a few Southeast Asian countries have officially adopted such schemes, including Malaysia, Thailand, Indonesia, Singapore, and Philippines. This highlights the need for the introduction and enforcement of GAP in Southeast Asian countries not only to meet regional food safety needs but to comply with stringent trade and export requirements.

Appropriate use and management of low-cost inputs and antimicrobials in agricultural production

Despite the apparent microbial hazards associated with the use of agricultural inputs such as fecal material and surface waters for fresh produce production, discontinuing the usage of these resources may not be feasible for some poor and vulnerable countries since they are high in nutrient content, relatively cheaper, and widely available. This calls for stringent quality assurance schemes to ensure the proper use and management of these resources. As an example, composting of raw fecal matter should be encouraged as this practice decomposes and stabilizes fecal organic material to produce a stable fertilizer applied to the soils (Malakahmad et al. 2017). Similarly, the treatment of contaminated surface waters for irrigation and the use of water sources with relatively good microbiological quality should be recommended to prevent the transfer of potentially pathogenic microorganisms from irrigation water to fresh produce (Uyttendaele et al. 2015).

The extensive use of antimicrobials has provided several benefits in animal production, including increased agricultural productivity in Southeast Asia (Holloway et al. 2015; Nhung et al. 2016). However, it has also led to the emergence and dissemination of AMR in different environments, including in food products, such as fresh produce. Policies on the appropriate use of antimicrobials in agriculture are available in Southeast Asia; however, there are gaps in their implementation. These include the reluctance of governments to collect and share data on antimicrobial use and management in their countries out of fear that they may be blamed for any negative findings (Holloway et al. 2015). This calls for the strict implementation of existing policies to promote appropriate antimicrobial use and cooperation among countries to arrive at a holistic approach to tackle the problem of AMR.

Increased awareness of the risks associated with current agricultural practices

The lack of knowledge of the possible risks associated with current agricultural practices in Southeast Asia contributes to microbial contamination of fresh produce. Although indigenous farming knowledge in Asia is generally perceived as valuable and usually correct (Rambo 2009), it must be adapted to the modern agricultural landscape. For instance, farmers in Vietnam use animal fecal material as fertilizers as they were either not aware or not concerned about the health hazards of being in contact with fecal material (Phuc et al. 2006; Jensen et al. 2008). Furthermore, farmers perceived “smelly feces” as a serious health risk and dry and odorless fecal material as generally safe (Lam et al. 2015). Similarly, most consumers are unfamiliar with health risks related to fresh produce consumption because of the common perception that they were safe to eat (Scott et al. 2009).

Collectively, this calls for increasing awareness of better farm practices and increased recognition of the potential hazards linked with utilizing highly contaminated resources in agricultural production and risks associated with the consumption of fresh and minimally processed foods. Increasing awareness among farmers and consumers will lead to better practices and informed decisions toward sustainable production and safe consumption of fresh produce.

Table 2: Future prospects and opportunities to address the problem of microbial contamination of fresh produce in Southeast Asia

1.	Enhanced research on the microbiological quality and microbial contamination of fresh produce in Southeast Asia
2.	Introduction and strict implementation of good agricultural practices in Southeast Asian countries
3.	Appropriate use and management of low-cost agricultural inputs and antimicrobials in agricultural production
4.	Increased awareness and understanding of the potential risks of current agricultural practices in Southeast Asia

Surveillance and detection technologies

Surveillance and detection technologies to determine the microbiological quality of fresh produce are important to guarantee food safety. There are different methods used in determining these pathogens namely culture-based methods (plaque assay and integrated cell culture real-time quantitative polymerase chain reaction (RT-qPCR), next-generation sequencing (NGS) methods, immunological methods [enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay], biosensor methods (optical biosensor, electrochemical biosensors and mass-based biosensors) and nucleic-acid based methods (PCR, qPCR, RT-qPCR, random amplification of polymorphic DNA (RAPD)-PCR, loop mediated isothermal amplification (LAMP), oligonucleotide DNA microarray, etc). However, these methods must follow the proper aseptic technique and sample storage in order to be effective. Based on the review of Aladhadh (2023), culture-based techniques work well with nucleic acid techniques in identifying various pathogens in fresh produce.

Additionally, there are surveillance networks that developed countries established to detect, monitor, and prevent foodborne pathogens. The Foodborne Diseases Active Surveillance Network, or FoodNet, has been tracking trends for infections transmitted commonly through food since 1996 in the United States. FoodNet provides a foundation for food safety policy and prevention efforts. It estimates the number of foodborne illnesses, monitors trends in incidence of specific foodborne illnesses over time, attributes illnesses to specific foods and settings, and disseminates this information. It is a collaborative project among Centers for Disease Control and Prevention (CDC), the 10 Emerging Infections Program sites, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) (FoodNet 2023).

Example of activities of FoodNet include conducting statewide surveillance such as in Connecticut, USA, active laboratory-based surveillance for bacterial and parasitic foodborne pathogens on every laboratory-diagnosed case of bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157:H7 and other non-O157 Shiga toxin-producing *E. coli* (STEC), *L. monocytogenes*, *Yersinia enterocolitica*, *Vibrio*, and the parasitic organism causing *Cyclospora* infection. This information is transmitted electronically to CDC. In addition to collecting laboratory-diagnosed cases of foodborne pathogens, investigators at FoodNet sites conduct active surveillance for hemolytic uremic syndrome (HUS) (a serious complication of STEC infection). The result is a comprehensive and timely database of foodborne illness in a well-defined population (FoodNet 2023).

On the other hand, Europe surveillance networks dealt with specific pathogens and used the One Health approach. For instance, European Network for Foodborne Parasites in Europe (EURO-FBP) aims to decrease the impact on human health from foodborne parasites (FBP), through establishing a risk-based control programme for FBP containing robust and appropriate protective strategies. EURO-FBP used an interdisciplinary, One Health perspective to assimilate information, coordinate research and harmonize diagnostics, surveillance, analytical methods, potential interventions and mapping of global trends regarding FBP (EURO-FBP 2022).

Further, another network, the One Health European Joint Program (OHEJP) MATRIX Project include activities which are focused on three specific pathogens—*Campylobacter*, *L. monocytogenes*, and *Salmonella*—in addition to a nonspecific hazard track on emerging threats (Henderson 2022).

CONCLUSIONS

Fresh produce in Southeast Asian countries is contaminated by microorganisms, and this is compounded by the presence of ARB. Several factors contributing to microbial contamination of fresh produce in Southeast Asia have been implicated. However, certain agricultural practices in the region, such as the use of low-cost agricultural inputs and extensive use of antimicrobials in agriculture, have been identified as the most likely causes. This indicates that the consumption of fresh produce in some Southeast Asian countries may be a potential health risk to consumers. This necessitates that proactive and urgent actions are taken to ensure the safety and integrity of fresh produce available to the public. These include enhanced research on the microbiological quality and microbial contamination of fresh produce, appropriate use of low-cost agricultural inputs and antimicrobials in agriculture, implementation of good agricultural practices, and heightened awareness, and understanding of potential risks of current agricultural practices among farmers and consumers in Southeast Asia (Table 2).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

PG Vital and WL Rivera gathered materials, wrote and reviewed the manuscript. Both authors are greatly involved in microbiological contamination studies in Philippine agricultural produce, products, and sources.

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