

# Antibiotic-Resistant Bacteria and Antibiotic Residue Contamination in Fresh Raw Foods Sold at Wholesale Markets in Thailand

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**Objective:** To investigate the extent of antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods sold at wholesale markets in Thailand, which may be the important drivers of antibiotic-resistant bacteria colonization and antibiotic-resistant bacterial infection in Thai population.

**Materials and Methods:** Fresh raw foods, including food from animal products, seafoods, vegetables, fruits, and honey were purchased from two large wholesale markets in Thailand. Food samples were cultured for antibiotic-resistant bacteria and tested for the presence and amount of antibiotic residue.

**Results:** Among 521 samples for bacterial culture, 86.9% grew at least one kind of bacteria. Enterobacteriaceae were commonly isolated and were commonly resistant to ampicillin (76.7% to 100%). ESBL-producers and ceftriaxone-resistant Enterobacteriaceae were prevalent in swine and duck samples (56.7% to 91.7%). Some isolates were resistant to co-amoxiclav (13.3% to 60.0%) and cefoxitin (5.0% to 30.0%). Colistin-resistant Enterobacteriaceae were observed in pork meat (1.4%) and chicken offal (7.0%). Ertapenem-resistant Enterobacteriaceae were detected in cha-om (26.7%). Among 501 samples for antibiotic residue testing, 37.1% contained at least one antibiotic residue. Enrofloxacin was the most prevalent antibiotic residue, followed by doxycycline and tilmicosin. Although most samples contained less antibiotics than the maximum residue limit (MRL), 7.0% contained an amount of at least one antibiotic above the MRL.

**Conclusion:** Many fresh raw foods sold at wholesale markets in Thailand were contaminated with antibiotic-resistant bacteria, and some contained antibiotic residues. Therefore, Thai people are at risk of being colonized with antibiotic-resistant bacteria and developing antibiotic-resistant bacterial infection due to consuming foods contaminated with antibiotic-resistant bacteria or containing antibiotic residues.

**Keywords:** antibiotic-resistant bacteria, antibiotic residue, fresh raw foods, wholesale market, Thailand

Received 3 December 2020 | Revised 26 January 2021 | Accepted 26 January 2021

J Med Assoc Thai 2021;104(4): 654-62

Website: <http://www.jmatonline.com>

Antimicrobial resistance (AMR) exerts massive adverse impact on human health and well-being in terms of morbidity, mortality, economic loss, animal health, food security, environmental well-being, and socioeconomic development<sup>(1,2)</sup>. The reports on AMR burden on human health in Thailand also revealed an enormous health and economic burden<sup>(3-6)</sup>. The World Health Organization (WHO), the World

Organisation for Animal Health (OIE), and the Food and Agriculture Organization of the United Nations (FAO) endorsed a global action plan to combat AMR in 2015<sup>(7)</sup>. The global action plan on AMR includes five strategic objectives aimed at ensuring the world's continued ability to treat and prevent infections with effective and safe medicines that are quality-assured, used in a responsible way, and accessible to all who need them<sup>(7)</sup>.

The Thailand AMR Containment and Prevention Program, which was established in 2012, analyzed the trend of AMR in Thailand and proposed a list of antimicrobial-resistant bacteria that pose an urgent threat to Thai people<sup>(8)</sup>. That list includes extended spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae, carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and carbapenem-resistant Enterobacteriaceae (CRE), and all those bacteria are also included in the WHO global priority list of critical antibiotic-resistant bacteria to guide research, discovery, and development of

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## How to cite this article:

Tangkoskul T, Thamthaweechok N, Seenama C, Thamlikitkul V. Antibiotic-Resistant Bacteria and Antibiotic Residue Contamination in Fresh Raw Foods Sold at Wholesale Markets in Thailand. J Med Assoc Thai 2021; 104:654-62.

[doi.org/10.35755/jmedassocthai.2021.04.12327](https://doi.org/10.35755/jmedassocthai.2021.04.12327)

new antibiotics announced by the WHO in February 2017<sup>(9)</sup>. ESBL-producing Enterobacteriaceae are the most common antibiotic-resistant bacteria that cause community-acquired infection, and they are also a common cause of healthcare-associated infection in Thailand<sup>(10-13)</sup>.

One of the risk factors for infection caused by ESBL-producing Enterobacteriaceae is having ESBL-producing Enterobacteriaceae colonization in the gastrointestinal tract<sup>(14,15)</sup>. The prevalence of fecal carriage of ESBL-producing Enterobacteriaceae was found to be much higher among people living in South East Asia than among those living in many countries in other regions of the world. The fecal carriage rate of ESBL-producing Enterobacteriaceae among South East Asians was less than 10% in 2002 but increased to 70% in 2011<sup>(16)</sup>. Colonization with ESBL-producing Enterobacteriaceae in the gastrointestinal tract of an individual can be caused by two main mechanisms. Endogenous mechanism is mediated by consumption of antibiotic or food containing antibiotic residue that could induce commensal bacteria, including Enterobacteriaceae in the gastrointestinal tract, to become resistant to antibiotics. Exogenous mechanism is mediated by direct consumption of or contact with antibiotic-resistant bacteria. The prevalence of fecal carriage of ESBL-producing Enterobacteriaceae in Thai people was reported to be higher than 50% in many communities<sup>(17-21)</sup>. Fecal carriage of ESBL-producing Enterobacteriaceae in these individuals is most likely associated with consumption of antibiotic, and consumption of food contaminated with ESBL-producing Enterobacteriaceae. The aforementioned factors should also be contributing to a high prevalence of fecal carriage of ESBL-producing Enterobacteriaceae among people who have traveled to South East Asia<sup>(22-26)</sup>. Fecal carriage of antibiotic-resistant bacteria is important because carriers can transmit those antibiotic-resistant bacteria to others, and they are at risk for developing antibiotic-resistant bacterial infection that is difficult to treat and has higher mortality than antibiotic-susceptible bacterial infection<sup>(12,27,28)</sup>.

The aim of the present cross-sectional study was to investigate the extent of antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods sold at wholesale markets in Thailand.

## Materials and Methods

Human or animal ethical consideration and approval were not required for this cross-sectional study.

## Fresh raw food samples

The fresh raw foods evaluated in the present study were purchased at the two largest wholesale markets in Thailand that sell fresh raw food for in-country consumption. The studied foods included food animal products from pork meat, pork liver, pork intestine, chicken meat, chicken offal, chicken egg, duck meat, duck offal, and duck egg, seafood as shrimp and tilapia fish, vegetables as bean sprout, Chinese morning glory, cha-om, ginger, coriander, spring onion, basil, and tomato, fruits as tangerine, pomelo, and lime, and honey. The study foods were purchased from both wholesale markets at 5:00 am on different days of the week between September 2017 and May 2018. The same study foods were purchased from different fresh raw food shops or fresh raw food trucks at both wholesale markets. One kilogram of each sample of most fresh raw foods was purchased. Five hundred grams of each sample was used for bacterial culture to determine the presence of antibiotic-resistant bacteria, and the other 500 grams of the same sample was used to detect antibiotic residue. Five hundred grams of some fresh raw food samples were purchased if that sample was going to be used only for detection of antibiotic residue. The purchased fresh raw food samples were packaged and taken away in the same type of container normally used by the seller to conduct normal business. The purchased samples were transported to the laboratory for bacterial culture within two hours after purchase, and they were maintained at 4°C until they were cultured within several hours. The samples purchased for detection of antibiotic residue were sent to the laboratory within six hours after purchase, and they were maintained at 4°C until they were processed for detection of antibiotic residue.

## Culture of fresh raw food samples for antibiotic-resistant bacteria

Three hundred grams of each fresh raw food sample was put into a blender device, and then 300 mL of tryptic soy broth (TSB) was added before blending the food sample for 10 minutes. The blended food sample was then incubated at 35°C for four hours before 10 µL of sample was used for bacterial culture in three types of solid agar, as follows, MacConkey agar supplemented with ceftriaxone 1 mg/L for screening Gram-negative bacteria resistant to ceftriaxone, mannitol salt agar for detection of *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), and enterococcosel agar supplemented with vancomycin 8 mg/L for detection of vancomycin-

**Table 1.** Type and prevalence of Gram-negative bacteria grown on MacConkey agar supplemented with ceftriaxone 1 mg/L

Type of Gram-negative bacteria	n (%)
Enterobacteriaceae	769 (93.7)
<i>Escherichia coli</i>	465 (60.5)
<i>Klebsiella pneumoniae</i> and other <i>Klebsiella</i> spp.	225 (29.3)
Others ( <i>Enterobacter</i> spp., <i>Citrobacter</i> spp., <i>Morganella</i> spp., <i>Edwardsiella</i> spp.)	79 (10.2)
Non-fermenting Gram-negative bacteria	52 (6.3)

resistant enterococci (VRE). The bacterial colonies grown on the agar plates were identified up to species level for Enterobacteriaceae, *S. aureus*, and VRE, and they were tested for antimicrobial susceptibility by disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI). The antibiotics tested against isolated Enterobacteriaceae were ampicillin, co-amoxiclav, ceftriaxone, cefoxitin, ertapenem, and colistin. Evaluation for the presence of ESBL of Enterobacteriaceae grown on MacConkey agar supplemented with ceftriaxone was performed by double-disk synergy test using ceftriaxone disk (30 µg), ceftazidime disk (30 µg), and amoxicillin or clavulanic acid disk (2:1, 30 µg).

#### Detection of antibiotic residue in fresh raw food samples

Food samples (500 mg for each sample) for antibiotic residue testing were sent to Central Laboratory (Thailand) Company Limited for analysis. This company has ISO/IEC 17025 certification for testing foods and agricultural products for quality and safety, and to act as a fast one-stop service for testing and certifying food products and commodities for export. The samples were tested for residue of ampicillin, amoxicillin, colistin, norfloxacin, enrofloxacin, oxytetracycline, chlortetracycline, doxycycline, ceftriaxone, ceftiofur, tiamulin, tilmosin, and tylosin by liquid chromatography-mass spectrometry (LC-MS/MS). The maximum residue limit (MRL) of each antibiotic for each kind of food is based on the values described in the announcements of the Department of Livestock Development (Thailand), the Department of Fisheries (Thailand), and CODEX ALIMENTARIUS International food standards on MRLs and risk management recommendations for residues of veterinary drugs in foods (CX/MRL 2-2018).

#### Data analysis

Antibiotic-resistant bacteria contamination and antibiotic residue in fresh raw food samples data

were described using descriptive statistics including frequency, percentage and 95% confidence interval.

## Results

### Antibiotic-resistant bacteria in 521 fresh raw food samples

Among 521 study samples for bacterial culture, 453 samples (86.9%) grew at least one kind of bacteria, with a total of 821 isolates of Gram-negative bacteria grown on MacConkey agar supplemented with ceftriaxone 1 mg/L. Most of those isolates (93.7%) were Enterobacteriaceae. The types of isolated Enterobacteriaceae are shown in Table 1. Most of the Enterobacteriaceae were *Escherichia coli* (60.5%), followed by *Klebsiella pneumoniae* and other *Klebsiella* spp. (29.3%). Nineteen isolates of coagulase-negative staphylococci (2.3%) were detected on mannitol salt agar. No bacterial growth was observed on enterococcosel agar supplemented with vancomycin 8 mg/L. Nine of 12 samples of honey grew only non-fermenting Gram-negative bacteria. No bacteria were detected from the content of chicken egg samples.

The prevalence of antibiotic resistance in Enterobacteriaceae isolated from study samples are shown in Table 2. Most isolates of Enterobacteriaceae were resistant to ampicillin (76.7% to 100%). ESBL-producers and ceftriaxone-resistant Enterobacteriaceae were prevalent in many raw food samples, especially pork and duck samples (56.7% to 91.7%). Some isolates of Enterobacteriaceae from study samples were resistant to co-amoxiclav (13.3% to 60.0%) and cefoxitin (5.0% to 30.0%). Colistin-resistant Enterobacteriaceae were observed in pork meat (1.4%) and chicken offal (7.0%). Ertapenem-resistant Enterobacteriaceae were detected only in cha-om samples (26.7%). No MRSA or VRE was isolated in any study samples.

### Antibiotic residue in 501 fresh raw food samples

Measurement of the amount of antibiotic residue in 501 fresh raw food samples revealed the detection

**Table 2.** Prevalence of antibiotic resistance in Enterobacteriaceae isolated from 521 study food samples

Food types	ESBL-E	Ampicillin-RE	Co-amoxiclav-RE	Ceftriaxone-RE	Cefoxitin-RE	Ertapenem-RE	Colistin-RE
Pork meat (n=70)	80.0%	100%	52.8%	82.8%	12.9%	0.0%	1.4%
Pork liver (n=29)	82.8%	96.6%	27.6%	82.8%	13.8%	0.0%	0.0%
Pork intestine (n=29)	79.3%	100%	20.7%	79.3%	13.8%	0.0%	0.0%
Chicken meat (n=60)	25.0%	100%	38.3%	25.0%	20.0%	0.0%	0.0%
Chicken offal (n=29)	34.5%	100%	31.1%	37.9%	27.6%	0.0%	7.0%
Duck meat (n=20)	56.7%	95.0%	60.0%	50.0%	20.0%	0.0%	0.0%
Duck offal (n=12)	91.7%	100%	33.3%	91.7%	16.7%	0.0%	0.0%
Shrimp (n=30)	50.0%	90.0%	33.3%	60.0%	13.3%	0.0%	0.0%
Tilapia fish (n=30)	6.7%	96.7%	30.0%	6.7%	30.0%	0.0%	0.0%
Bean sprout (n=43)	44.2%	86.1%	44.2%	46.5%	16.3%	0.0%	0.0%
Chinese morning glory (n=14)	0.0%	100%	50.0%	50.0%	0.0%	0.0%	0.0%
Cha-om (n=30)	43.3%	90.0%	26.7%	36.7%	23.3%	26.7%	0.0%
Coriander (n=40)	20.0%	90.0%	17.5%	20.0%	5.0%	0.0%	0.0%
Spring onion (n=30)	3.3%	76.7%	26.7%	6.7%	20.0%	0.0%	0.0%
Basil (n=30)	13.3%	90.0%	13.3%	13.3%	20.0%	0.0%	0.0%
Tomato (n=25)	0.0%	80.0%	40.0%	28.0%	0.0%	0.0%	0.0%

ESBL-E= extended spectrum  $\beta$ -lactamase-producing Enterobacteriaceae; Ampicillin-RE=ampicillin-resistant Enterobacteriaceae; Co-amoxiclav-RE=co-amoxiclav-resistant Enterobacteriaceae; Ceftriaxone-RE=ceftriaxone-resistant Enterobacteriaceae; Cefoxitin-RE=cefoxitin-resistant Enterobacteriaceae; Ertapenem-RE=ertapenem-resistant Enterobacteriaceae; Colistin-RE=colistin-resistant Enterobacteriaceae

of ampicillin, norfloxacin, enrofloxacin, doxycycline, oxytetracycline, chlortetracycline, ceftriaxone, and tilmicosin: however, amoxicillin, colistin, ceftiofur, tiamulin, and tylosin were not detected. Among the 501 samples, 186 samples (37.1%) contained at least one tested antibiotic. Among those 186 study samples, 140 samples (75.3%) contained only one antibiotic, 39 samples contained two antibiotics (21.0%), and seven samples (3.8%) contained three antibiotics. Among the 186 study samples with detectable antibiotics, 13 samples (7.0%) contained an amount of at least one antibiotic above the MRL. However, 43 of 186 samples (23.1%) contained antibiotics with an unavailable MRL, or the detected antibiotics were not authorized for use in the foods in which they were found, such as norfloxacin or ceftriaxone in food animals, and ampicillin in tangerines.

The prevalence of the presence of antibiotic in each type of study food is shown in Table 3. The tested antibiotic residues were commonly detected in fresh raw food animals, including fish, in 8.0% to 65.5% of samples. Antibiotic residue was also detected in 18.6% of tangerine samples. There was no detection of any of the tested antibiotics in any of the duck egg, honey, cha-om, ginger, pomelo, or lime samples.

The amount of various antibiotics detected in study samples with detectable antibiotic residue

**Table 3.** Prevalence of the presence of antibiotic in 501 study raw food samples

Type of raw food sample	Number of samples containing at least one antibiotic; n (%)
Pork meat (n=65)	34 (52.3)
Pork liver (n=29)	18 (62.1)
Pork intestine (n=29)	19 (65.5)
Chicken meat (n=55)	35 (63.6)
Chicken offal (n=29)	14 (48.3)
Chicken egg (n=63)	23 (36.5)
Duck meat (n=20)	11 (55.0)
Duck egg (n=10)	0 (0.0)
Shrimp (n=25)	2 (8.0)
Tilapia fish (n=31)	17 (54.8)
Tangerine (n=70)	13 (18.6)
Pomelo (n=30)	0 (0.0)
Lime (n=20)	0 (0.0)
Honey (n=15)	0 (0.0)
Cha-om (n=5)	0 (0.0)
Ginger (n=5)	0 (0.0)

are shown in Table 4. Enrofloxacin was the most prevalent antibiotic residue detected in study samples, followed by doxycycline and tilmicosin. Norfloxacin

**Table 4.** Range of amount of each antibiotic in 186 study fresh raw food samples with detectable antibiotic residue

Food type	Ampicillin (µg/kg)	Norfloxacin (µg/kg)	Enrofloxacin (µg/kg)	Doxycycline (µg/kg)	Oxytetracycline (µg/kg)	Chlortetracycline (µg/kg)	Ceftriaxone (µg/kg)	Tilmicosin (µg/kg)
Pork meat (n=34)	13.6	<0.8 to 243.1	<0.8 to 243.1	30.0	39.6	<20.0 to 240.0	UD	<15.0 to 190.0
Pork liver (n=18)	UD	UD	<0.8 to 1,932.5	<20.0 to 338.6	UD	182.0	UD	<15.0 to 534.1
Pork intestine (n=19)	UD	2.4 to 56.0	2.9 to 58.3	UD	UD	<20.0 to 2,469.0	UD	8.1 to 160.0
Chicken meat (n=35)	UD	UD	<0.8 to 164.5	<20.0 to 42.0	UD	UD	UD	UD
Chicken offal (n=14)	UD	UD	<0.8 to 26.5	<20.0 to 34.9	UD	UD	UD	<15.0 to 63.0
Chicken egg (n=23)	UD	UD	0.5 to 3.1	<20.0 to 52.8	UD	UD	50.0 to 210.0	UD
Duck meat (n=11)	UD	UD	0.4 to 1.0	UD	UD	UD	UD	UD
Shrimp (n=2)	UD	UD	1.3 to 14.8	UD	UD	UD	UD	UD
Tilapia fish (n=17)	UD	UD	0.5 to 2.9	UD	UD	UD	20.0 to 30.0	UD
Tangerine (n=13)	3.5 to 235.3	UD	UD	UD	UD	UD	UD	UD

UD=undetectable

and ceftriaxone, which are important antibiotics for human health, were detected in some study samples. Although most of the study samples contained antibiotic residues less than the MRL, 7.0% of study samples contained the amount of at least one antibiotic more than the MRL. Some study samples contained a much higher concentration of antibiotics than the MRL for that antibiotic (e.g., 1,932.5 µg/kg of enrofloxacin and 2,469.0 µg/kg of chlortetracycline).

## Discussion

Fresh raw foods sold at wholesale markets were used in the present study because they are normally purchased directly from fresh raw food producers from many different provinces in Thailand. The people that shop in wholesale markets range from individual consumers to restaurant and store owners. The fresh raw foods selected for the present study were chosen for several reasons. The main reason is that most of the selected food items are commonly consumed by Thai people. The selected vegetables are usually consumed as raw foods without cooking by heat. Farmers in Thailand use antibiotic for prevention and treatment of citrus greening disease in tangerine and other citrus fruits. Honey was reported to contain antibiotic<sup>(29)</sup>. The authors did not culture some kinds of food samples, such as tangerine, because people do not consume the skin of tangerine. The present study focused on the antibiotic-resistant bacteria that are common or important for causing community-acquired colonization or infections in Thai people, especially ceftriaxone-resistant Enterobacteriaceae and ESBL-producing Enterobacteriaceae. Therefore, the authors needed to screen the contaminated bacteria in study samples with agars supplemented with

antibiotics to improve the ability to identify antibiotic-resistant bacteria contamination. The antibiotic residues tested in study samples were selected based on information on the types of antibiotic that are commonly used on food animal farms, in fruits, and they are important in human health, such as ceftriaxone. The authors did not test antibiotic residues in some food samples, such as vegetables, because the authors had information that antibiotic was not used for growing vegetables. The present study was unable to test many more antibiotic residues because the cost of detecting each antibiotic residue in each food sample is quite high, and the present study had a limited budget. Therefore, the antibiotic residues found in food samples in the present study should be considered non-comprehensive because the food samples in the present study might have contained additional antibiotic residues that were not tested.

The present study results revealed a very high prevalence of contamination of antibiotic-resistant bacteria, especially ceftriaxone-resistant Enterobacteriaceae and ESBL-producing Enterobacteriaceae, in many fresh raw foods sold at the selected wholesale markets in Thailand. MRSA and VRE were not detected in any food sample because these antibiotic-resistant bacteria are usually detected in hospitals, and they are rarely presented in community. Although contamination of antibiotic-resistant bacteria in the fresh raw foods sold at the present study wholesale markets could occur during transportation from the production site to the wholesale market or during storage prior to sale, it is most likely that antibiotic-resistant bacteria contamination would occur at the production site for several reasons. The prevalence of antibiotic-resistant bacteria contamination in many



study foods, especially in food animal products and in some of the vegetables tested in the present study, was similar to that found in samples collected from food animal farms and retail shops in the previous studies<sup>(19,20)</sup>. Moreover, the prevalence of antibiotic-resistant bacteria contamination in samples of the same kind of food purchased from different shops and trucks was variable even though the process of transportation and storage of these foods were generally similar among different types of wholesale vendors. Antibiotic-resistant bacteria contamination in food from animal products is, therefore, most likely attributable to the pressure effect of antibiotic use for prevention or treatment of infection in food animals on the bacteria residing in food animals and in the farm environment to become antibiotic-resistant. Antibiotic-resistant bacteria contamination in some kinds of vegetables could be associated with the use of fertilizer made from feces of food animals or from wastewater that contains antibiotic-resistant bacteria<sup>(19,30,31)</sup>. Some of the foods evaluated in the present study were contaminated with uncommon, but very important antibiotic-resistant bacteria such as colistin-resistant and CRE, and this should be of particular concern since infections caused by these antibiotic-resistant bacteria are difficult to treat and they are associated with high mortality. CRE contamination in cha-om, which is a kind of vegetable, is interesting and the authors are investigating how this kind of vegetable becomes contaminated with CRE. It should be mentioned that cha-om exported from Thailand to Switzerland was contaminated with ESBL-producing and colistin-resistant Enterobacteriaceae<sup>(32)</sup>. The consumers of properly heat-cooked foods should be safe from live antibiotic-resistant bacteria, but antibiotic-resistant genes are usually heat-stable, and they might transfer antibiotic resistance to the bacteria in the gastrointestinal tract of the consumer.

The present study results also revealed a high prevalence of antibiotic residue in fresh raw foods sold at the selected wholesale markets in Thailand. One-third of samples with identified antibiotic residue contained more than one kind of antibiotic. Ceftriaxone and norfloxacin, which are important antibiotics for human health and not permitted for use in food production, were detected in some study samples. Ampicillin was detected in tangerine even though it has never been recommended for use in tangerine production. These observations indicate that antibiotics are extensively used in food production, and the withdrawal period after antibiotic use in food

production was too short, especially in the foods that contained a very high amount of antibiotics. It is worth noting that enrofloxacin, which is a member of the fluoroquinolone family of broad-spectrum antibiotics reported to be associated with the development of multidrug-resistant Enterobacteriaceae<sup>(33)</sup>, was the most common antibiotic residue found in the food samples in the present study. Colistin is commonly used in swine, but all study samples of swine products had no detectable colistin residue. This observation is likely due to the fact that colistin is poorly absorbed after ingestion and it is usually used in swine aged less than one month. Therefore, its non-detectability can be explained by the 5-month withdrawal period between the use of the first month of life and the 6-month time point when swine are normally sent for slaughter. Although most of the food samples with detectable antibiotics contained an amount of antibiotics below the MRL, only a small amount of antibiotic is needed to induce antibiotic resistance in bacteria<sup>(34-36)</sup>. It should be emphasized that at least 7% of study samples contained an amount of antibiotic above the MRL, and these foods should not be sold or consumed.

The antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods observed in the present study poses a health threat to consumers because consumers cannot differentiate between fresh raw foods with or without antibiotic-resistant bacteria or antibiotic residue by their general appearance. Therefore, consumers unknowingly purchase and consume these contaminated foods will acquire antibiotic-resistant bacteria or antibiotic residue. Even though many study foods, especially food from animal products, are usually consumed after cooking by heat, consumers are still at risk of acquiring antibiotic-resistant bacteria and antibiotic residue. Antibiotic-resistant bacteria contamination in these raw foods can be transmitted to other raw foods that are freshly consumed without cooking by heat such as fresh vegetables via cross-contamination. For example, a chopping block that is used to prepare different kinds of foods can facilitate the transmission of antibiotic-resistant bacteria from a food that is contaminated to a food that is not. Moreover, many antibiotics, especially enrofloxacin, are heat stable<sup>(37)</sup>, so these antibiotic residues can also induce antibiotic resistance in bacteria residing in gastrointestinal tract of the consumer even after the foods containing such antibiotics are cooked by heat. Therefore, consumption of foods contaminated with antibiotic-resistant bacteria or antibiotic residue

should be the main risk factor for being a carrier of antibiotic-resistant bacteria in the gastrointestinal tracts of Thai people and travelers, and these carriers are at risk of developing a subsequent infection caused by antibiotic-resistant bacteria.

The strategies to minimize antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods include 1) food producers avoid the use of antibiotics in food production, 2) if antibiotic use is essentially necessary for food production, it should be used appropriately relative to type, dose, route and, duration of antibiotic, 3) if antibiotic is used for food production, the withdrawal period after antibiotic use should be long enough to ensure that no antibiotic residue is present in food prior to sale, 4) responsible regulatory authority should continuously enforce and reinforce the laws, regulations, or recommendations for the use of antibiotic for food production, 5) responsible regulatory authority should continuously monitor for antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods and provide corrective measures, and 6) a rapid test for detection of antibiotic-resistant bacteria and antibiotic residue that can be used by the consumer at the site of food purchase should be developed. Food consumers should be educated about the risks of purchasing foods contaminated with antibiotic-resistant bacteria or antibiotic residue, they should mindfully attempt to purchase only antibiotic-free foods and should consume appropriately heat-cooked foods and clean raw foods.

The main limitation of the present study is that, compared to the number of samples for most food types, there was a smaller number of samples for some other food types for determination of antibiotic-resistant bacteria and antibiotic residue. Therefore, the prevalence of antibiotic-resistant bacteria and antibiotic residue contamination in those less well represented food types could be more or less than the results observed in the present study.

In conclusion, fresh raw foods sold at selected wholesale markets in Thailand were commonly found to be contaminated with antibiotic resistant bacteria, and some of them contained antibiotic residues. Therefore, Thai people are at risk of being colonized by antibiotic-resistant bacteria and developing antibiotic-resistant bacterial infection due to consuming foods contaminated with antimicrobial-resistant bacteria or containing antibiotic residues. Strategies to minimize antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods

are to increase awareness of the risk of contamination by antibiotic-resistant bacteria and antibiotic residue in fresh raw foods, and to promote appropriate food consumption behavior.

### **What is already known on this topic?**

Prior reports on contamination of antibiotic-resistant bacteria and antibiotic residue in foods in Thailand were scattered and they were not determined in the same food samples.

### **What this study adds?**

The same fresh raw foods sold at wholesale markets in Thailand are determined for contamination of antibiotic-resistant bacteria and antibiotic residue. Many collected fresh raw food samples are contaminated with antibiotic-resistant bacteria and some fresh raw foods contain antibiotic residues. Therefore, Thai people are at risk of being colonized with antibiotic-resistant bacteria and developing antibiotic-resistant bacterial infection due to consuming foods contaminated with antibiotic-resistant bacteria or containing antibiotic residues.

### **Acknowledgement**

The authors gratefully acknowledge Mr. Nuttapon Srasrisom and Ms. Wanida Cheewathammarat for their assistance with purchasing the study food samples, secretarial management, and coordination with the funding agency.

### **Funding disclosure**

The present work was supported by the Agricultural Research Development Agency, Thailand to VT [grant no. POP5905021420]. The funding body was not involved in the design or execution of the study, the analyses of the study results, or the preparation of the manuscript.

### **Conflicts of interest**

All authors declare no personal or professional conflicts of interest relating to any aspect of the present study.

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