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Research article

Occurrence and molecular characteristics of antimicrobial resistance, virulence factors, and extended-spectrum β-lactamase (ESBL) producing *Salmonella enterica* and *Escherichia coli* isolated from the retail produce commodities in Bangkok, Thailand

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ABSTRACT

The incidence of antimicrobial resistance (AMR) in the environment is often overlooked and leads to serious health threats under the One Health paradigm. Infection with extended-spectrum β-lactamase (ESBL) producing bacteria in humans and animals has been widely examined, with the mode of transmission routes such as food, water, and contact with a contaminated environment. The purpose of this study was to determine the occurrence and molecular characteristics of resistant *Salmonella enterica* (*S. enterica*) ($n = 59$) and *Escherichia coli* (*E. coli*) ($n = 392$) isolated from produce commodities collected from fresh markets and supermarkets in Bangkok, Thailand. In this study, the *S. enterica* isolates exhibited the highest prevalence of resistance to tetracycline (11.9%) and streptomycin (8.5%), while the *E. coli* isolates were predominantly resistant to tetracycline (22.5%), ampicillin (21.4%), and sulfamethoxazole (11.5%). Among isolates of *S. enterica* (6.8%) and *E. coli* (15.3%) were determined as multidrug resistant (MDR). The prevalence of ESBL-producing isolates was 5.1% and 1.0% in *S. enterica* and *E. coli*, respectively. A minority of *S. enterica* isolates, where a single isolate exclusively carried $bla_{\text{CTX-M-55}}$ (n = 1), and another isolate harbored both $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-1}}$ (n = 1); similarly, a minority of *E. coli* isolates contained $bla_{\text{CTX-M-55}}$ ($n = 2$) and $bla_{\text{CTX-M-15}}$ ($n = 1$). *QnrS* (11.9%) and bla_{TEM} (20.2%) were the most common resistant genes found in *S. enterica* and *E. coli,* respectively. Nine isolates resistant to ciprofloxacin contained point mutations in *gyrA* and *parC*. In addition, the odds of resistance to tetracycline among isolates of *S. enterica* were positively associated with the co-occurrence of ampicillin resistance and the presence of *tetB* ($P = 0.001$), while the *E. coli* isolates were positively associated with ampicillin resistance, streptomycin resistance, and the presence of *tetA* ($P < 0.0001$) in this study. In summary, these findings demonstrate that fresh vegetables and fruits, such as cucumbers and tomatoes, can serve as an important source of foodborne AMR *S. enterica* and *E. coli* in the greater Bangkok area, especially given the popularity of these fresh commodities in Thai cuisine.

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1. Introduction

Salmonella enterica (*S. enterica*) and *Escherichia coli* (*E. coli*) have been implicated in numerous foodborne disease outbreaks associated with consumption of contaminated fresh produce such as leafy greens, basil, sprouts, cucumber, and lettuce [1–3]. These bacterial pathogens are important targets for monitoring and surveillance of phenotypic and genotypic AMR not only in food-producing animals, but also for commonly consumed food items and in the environment such as drinking and recreational water [4,5]. An estimated 111,295 human cases and 48,258 deaths occur each year due to AMR infections in Thailand [6]. Furthermore, there are an estimated 9,400,000 human infections and 155,000 deaths worldwide per year caused by *Salmonella* spp [7]. In the US, although the prevalence of *S. enterica* in fresh produce is low, the presence of AMR *Salmonella* among multiple serotypes has been widely reported in fresh vegetables [8]. With respect to Southeast Asia, previous studies in Malaysia and Vietnam have documented MDR *S. enterica* in fresh vegetables, which were mainly resistant to ampicillin, tetracycline, nalidixic acid, trimethoprim-sulfamethoxazole, and chloramphenicol [9,10]. Similar observations have been made for AMR *E. coli* and produce; for example, commensal *E. coli* isolated from fresh vegetables exhibited a high prevalence of AMR to streptomycin (93%) and tetracycline (30%), with 21% of the isolates being MDR [11].

Of particular concern is the occurrence of extended-spectrum β-lactamase (ESBL)-producing bacteria in human or food producing animal, given that these enzymes confer resistance to penicillins and third- and fourth-generation cephalosporins, which are clinically important antibiotics for human and animal medicine [12]. The emergence of ESBL bacteria has been caused by the widespread circulation of plasmids containing different β-lactamases, including SHV-, TEM-, CTX-M, and Toho β-Lactamases, OXA, PER, VEB-1, BES-1 and other ESBLs [13]. In addition, quinolones are broad spectrum antibiotics that can be effective for the treatment of pathogenic *Enterobacteriaceae* infections. The new generation fluoroquinolones (FQs) are widely used in human and veterinary medicine, perhaps facilitating the concomitant rise in FQ resistance. One growing concern about FQ resistance is its association with ESBL production in *Enterobacteriaceae* [14], with mutations in the quinolone-resistance-determining regions (QRDRs), a common mechanism of quinolone resistance that can lead to complicated treatment of gram-negative bacterial infections. Resistance to quinolones is mainly caused by spontaneous point mutations that substitute amino acids in the QRDRs of either *gyrA* or *parC*, or both genes [15]. Monitoring and surveillance of ESBLs and QRDRs in bacterial contaminants in common foods such as fresh fruits and vegetables is an important strategy to reduce human foodborne infection with these resistant bacteria.

Although Thailand is a major producer of fresh fruits and vegetables, epidemiological data on phenotypic and genotypic AMR are very limited. Furthermore, much of Thai cuisine makes use of fresh, raw, or partially cooked produce items, leading to possible direct exposure to foodborne AMR bacteria like *S. enterica* and *E. coli*. Although contamination of AMR bacteria in fresh vegetables has been widely documented in Thailand [16–18], genetic characterization of virulence profiles, and semi-quantitative analysis of AMR phenotype are relatively limited. Therefore, the objectives of this study were to characterize the virulence factors, AMR profiles and their determinants, and to genetically characterize ESBL and QRDR mutation regions in *gyrA* and *parC* of *S. enterica* and *E. coli* isolated from fresh vegetables and fruits from the retail markets in Bangkok, Thailand.

Table 1
S. enterica (n = 59) and *E. coli* (n = 392) isolates obtained from produce commodities from open-air fresh markets and supermarkets throughout Bangkok, Thailand, 2018–2019.

Type of vegetable	No. of bacterial isolations			
	Supermarket		Fresh market	Total (n = 451)>
	Conventional	Organic	Conventional	
S. enterica				
Sweet basil	11	0	10	21
Spring onion	3	1	2	6
Coriander	1	2	10	13
Cabbage	0	0	0	0
Lettuce	4	0	12	16
Cucumber	0	0	3	3
Tomato	0	0	0	0
Total	19	3	37	59
E. coli				
Sweet basil	27	23	29	79
Spring onion	18	18	26	62
Coriander	23	22	28	73
Cabbage	5	2	27	34
Lettuce	25	18	28	71
Cucumber	11	7	26	44
Tomato	6	8	15	29
Total	115	98	179	392
Grand total	134	101	216	451

2. Materials and methods

2.1. Bacterial isolates

A total of 392 *E. coli* and 59 *S. enterica* isolates were previously obtained from a longitudinal epidemiological study conducting active surveillance for bacterial contamination of various produce commodities common in Thai cuisine (cabbage, coriander, cucumber, lettuce, spring onion, sweet basil, tomato) obtained from open-air fresh markets and enclosed supermarkets throughout Bangkok, Thailand, between May 2018 and February 2019 [19]. Fresh markets were defined as open-air retail operations in which fresh fruits and vegetables are sold in bulk and generally not packaged nor refrigerated; supermarkets were generally enclosed retail operations with temperature control and produce items packaged before retail display. Produce commodities were further classified as grown under organic or conventional farming conditions, as indicated by the retail label or self-reported by the fresh market vendor (Table 1). Sampling procedures, bacterial isolation and confirmation, *Salmonella* serotyping, and storage conditions of the isolates at the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University are fully described in a previous publication [19]. The methods of this study were carried out with relevant guidelines and regulations of Chulalongkorn University, the Institutional Biosafety Committee of the Faculty of Veterinary Committee (IBC 2231042). Briefly, all samples were purchased and separately packed in a sterile plastic bag using sterile conditions. During transportation the sample was maintained at < 10 °C in a cool box, then processed within 24 h after collection. Approximately, 25 g of each vegetable and 200 g of each fruit were added into Buffered Peptone Water (BPW) (Difco, MD, USA) to make a ten-fold serial dilution. For the isolation of *E. coli*, one mL of each BPW dilution was inoculated in nine mL lactose broth (Difco) and incubated at 37 °C overnight. Then, one loopful of the suspension was transferred to a nine mL EC broth (Difco) and incubated at 45.5 °C for 24 h. One loopful of positive EC broth was streaked on the Levine's Eosin-Methylene Blue (L-EMB) agar (Difco), incubated at 37 °C for 24 h, and biochemically confirmed using indole and Triple Sugar Iron (TSI) (Difco).

For the isolation of *Salmonella* spp., the inoculated suspension was transferred to Modified Semi-Solid Rappaport-Vassiliadis (MSRV) (Difco) agar and incubated at 42 °C for 24 h. One loopful of positive MSRV plates was streaked on Xylose Lysine Deoxycholate (XLD) (Difco) and Hektoen Enteric (HE) (Difco) agar plates, incubated at 37 °C and biochemically confirmed according to the U.S. FDA BAM [20]. *Salmonella* serotyping was performed using a slide agglutination test according to Kauffman and White scheme [21] with commercially available antisera (S&A Reagents Lab, Bangkok, Thailand). All *E. coli* and *S. enterica* isolates were kept in 20% glycerol and stored at −80 °C.

2.2. Antimicrobial susceptibility testing

All isolates of *S. enterica* and *E. coli* were tested for minimum inhibitory concentrations (MIC) using a two-fold agar dilution method against nine antimicrobial agents (the recommended clinical breakpoints and the concentration ranges are in parentheses): ampicillin (AMP, 32 μg/mL, 0.5–1024 μg/mL), chloramphenicol (CHP, 32 μg/mL, 1–256 μg/mL), ciprofloxacin (CIP, 1 μg/mL, 0.008–64 μg/mL), gentamicin (GEN, 8 μg/mL, 0.125–64 μg/mL), streptomycin (STR, 32 μg/mL, 1–512 μg/mL), sulfamethoxazole (SUL, 512 μg/mL, 4–4096 μg/mL), tetracycline (TET, 16 μg/mL, 0.125–256 μg/mL), trimethoprim (TRI, 16 μg/mL, 0.125–1024 μg/mL), and colistin (COL, 2 μg/mL, 0.125–128 μg/mL) according to the standard protocol [22].

Briefly, the bacterial isolates were grown on Muller-Hinton agar (MHA) (Difco, MD, USA) at 37 °C overnight. A bacterial suspension with turbidity equivalent to 0.5 McFarland was then prepared in a 0.85% NaCl solution, diluted 10-fold, and inoculated onto MHA containing the indicated concentrations of antimicrobial agents as described above. The inoculated MHA plates were incubated at 37 °C for 16–18 h. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Bacterial isolates that exhibited concurrent resistance at least one antimicrobial agent in three or more than three different classes of antimicrobials were considered MDR different classes of antimicrobials were considered MDR.

For carbapenems, the disk diffusion method was used for antimicrobial susceptibility screening for all isolates using imipenem (IPM, 10 μg) and meropenem (MEM, 10 μg) according to standard protocols [22]. Briefly, the concentration of bacteria was adjusted by 0.5 McFarland standard and the suspension inoculated in MHA using a sterile cotton swab. Antimicrobial disks were placed in inoculated plates, followed by incubation at 37 °C for 16–18 h. The clinical breakpoints of an inhibition clear zone for both imipenem and meropenem are at least 19 mm, with inhibition zones less than 19 mm defined as resistant strains. *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853 were used as quality control strains.

2.3. Determination of ESBL production

Disk diffusion was used to identify production of ESBLs for all isolates according to the Clinical & Laboratory Standards Institute [22]. Briefly, the concentration of bacteria was adjusted to a 0.5 McFarland standard and the suspension inoculated onto MHA using a sterile cotton swab. The initial screening of ESBL production was identified by disk diffusion using ceftazidime (30 μg), cefotaxime (30 μg), and cefpodoxime (10 μg) (Liofilchem, Roseto Degli Abruzzi, Italy). The antimicrobial disks were placed on inoculated plates, followed by incubation at 37 °C for 16–18 h. Isolates that produced an inhibition clear zone of at least 11 mm in diameter were defined as resistant strains. Isolates that showed resistance to at least one cephalosporin test were then subjected to ESBL phenotypic confirmation by a combination disk assay using cefotaxime (30 μg) and ceftazidime (30 μg) with and without clavulanic acid (10 μg) (Liofilchem). Isolates that showed an increase equal to and greater than 5 mm in the zone of inhibition in a combination disk were considered ESBL producers.

Table 2
PCR primer pairs used to identify AMR, ESBL, and virulence genes in *E. coli* and *S. enterica*.

Gene	Primer	Oligonucleotide sequences	Annealing (C)	Product size (bp)	Reference
Ampicillin					
bla_{TEM}	bla_{TEM}-F	ATCAGTTGGGTGCACGAGTG	60	608	[24]
	bla_{TEM}-R	ACGCTCACCGGCTCCAGA			
bla_{pse}	bla_{PSE}-F	GCTCGTATAGGTGTTTCCGTTT	60	575	[25]
	bla_{PSE}-R	CGATCCGCCGAHTGTTCCATCC			
Chloramphenicol					
catA	*catA*-F	CCAGACCGTTCAGCTGGATA	58	454	[26]
	catA-R	CATCAGCACCTTGTCGCCT			
cmlA	*cmlA*-F	TGGACCGCTATCGGACCG	57	641	[26]
	cmlA-R	CGCAAGACACTTGGGCTGC			
floR	*floR*-F	ATGGTGATGCTCGGCGTGGGCCA	58	800	[27]
	floR-R	GCGCCGTTGGCGGTAACAGACACCGTGA			
Tetracycline					
tetA	*tetA*-F	GCTGTCGGATCGTTTCGG	60	658	[26]
	tetA-R	CATTCCGAGCATGAGTGCC			
tetB	*tetB*-F	CTGTCGCGGCATCGGTCAT	60	615	[26]
	tetB-R	CAGGTAAAGCGATCCCACC			
tetW	*tetW*-F	ATGAACATTCCCACCGTTA	53	101	[28]
	tetW-R	ATATCGGCGGAGAGCT			
tetO	*tetO*-F	CAACATTAACGGAAAGTTT	53	104	[28]
	tetO-R	TTGACGCTCCAAATTCA			
tetT	*tetT*-F	CCATATAGAGGTTCCACCA	53	73	[28]
	tetT-R	TGACCCTATTGGTAGTG			
Trimethoprim					
dfrA1	*dfrA1*-F	GGAGTGCCAAAGGTGAACAGC	55	367	[29]
	dfrA1-R	GAGGCGAAGTCTTGGGTAAAAAC			
dfrA12	*dfrA12*-F	TTCGCAGACTCACTGAGGG	55	330	[26]
	dfrA12-R	CGGTTGAGACAAGCTCGAAT			
dfrA14	*dfrA14*-F	GATTGGTTGCGGTCCA	53	379	[30]
	dfrA14-R	CTCAAAAACAACTTCGAAGG			
Streptomycin					
strA	*strA*-F	TGGCAGGAGGAACAGGAGG	57	405	[26]
	strA-R	AGGTCGATCAGACCCGTGC			
strB	*strB*-F	GCGGACACCTTTTCCAGCCT	57	621	[26]
	strB-R	TCCGCCATCTGTGCAATGCG			
aadA	*aadA-F*	CCCCTGGAGAGAGCGAGATT	61	152	[31]
	aadA-R	CGTGGCTGGCTCGAAGATAC			
Gentamicin					
aadB	*aadB-F*	CCTGCTTGGTGGGCAGAC	55	244	[32]
	aadB-R	CGGCACGCAAGACCTC			
aphA-1	*aphA-1-F*	TGAACAAGTCTGGAAAGA	50	114	[28]
	aphA-1-R	CCTATTAATTTCCCCTC			
Ciprofloxacin					
qnrA	*qnrA*-F	AGAGGATTTCTCACGCCAGG	54	580	[33]
	qnrA-R	TGCCAGGCACAGATCTTGAC			
qnrB	*qnrB*-F	GGMATHGAAATTCGCCACTG	54	264	[33]
	qnrB-R	TTTGCYGYYCGCCAGTCGAAC			
qnrS	*qnrS*-F	GCAAGTTCATTGAACAGGGT	54	428	[33]
	qnrS-R	TCTAAACCGTCGAGTTCGGCG			
Sulfamethoxazole					
Sul1	*sul1*-F	CGGCGTGGGCTACCTGAACG	66	433	[34]
	sul1-R	GCCGATCGCGTGAAGTTCCG			
Sul2	*sul2*-F	CGGCATCGTCAACATAACCT	66	721	[34]
	sul2-R	TGTGCGGATGAAGTCAGCTC			
Sul3	*sul3*-F	CAACGGAAGTGGGCGTTGTGGA	66	244	[34]
	sul3-R	GCTGCACCAATTCGCTGAACG			
Colistin					
mcr-1	*mcr-1-F*	AGTCCGTTTGTTCTTGTGGC	58	320	[35]
	mcr-1-R	AGATCCTTGGTCTCGGCTTG			
mcr-2	*mcr-2-F*	CAAGTGTGTTGGTCGCAGTT	58	715	[35]
	mcr-2-R	TCTAGCCCGACAAGCATACC			
mcr-3	*mcr-3-F*	AAATAAAAATTGTTCCGCTTATG	58	929	[35]
	mcr-3-R	AATGGAGATCCCCGTTTTT			
mcr-4	*mcr-4-F*	TCACTTTCATCACTGCGTTG	58	1116	[35]
	mcr-4-R	TTGGTCCATGACTACCAATG			
mcr-5	*mcr-5-F*	ATGCGGTTGTCTGCATTTATC	58	1644	[35]
	mcr-5-R	TCATTGTGGTTGTCCTTTTCTG			
Carbapenem					

(*continued on next page*)

Table 2 (*continued*)

Gene	Primer	Oligonucleotide sequences	Annealing (C)	Product size (bp)	Reference
bla_{NDM}	bla_{NDM}-F	GGTTTGGCGATCTGGTTTTC	52	621	[36]
	bla_{NDM}-R	CGGAATGGCTCATCACGATC			
bla_{OXA}	bla_{OXA}-F	ACACAATACATATCAACTTCGC	62	813	[37]
	bla_{OXA}-R	AGTGTGTGTTTAGAATGGTGATC			
ESBL gene					
bla_{SHV}	bla_{SHV}-F	TTATCTCCCTGTTAGCCACC	50	797	[38]
	bla_{SHV}-R	GATTTGCTGATTTCGCTCGG			
bla_{CTX-M}	bla_{CTX-M}-F	CGATGTGCAGTACCAGTAA	60	585	[39]
	bla_{CTX-M}-R	AGTGACCAGAATCAGCGG			
Disinfectant genes					
qacE	*qacE-F*	CCCGAATTCATGAAAGGCTGGCTT	55	350	[40]
	qacE-R	TAAGCTTTCACCATGGCGTCGG			
Integrons					
Int1	*int1*-F	CCTGCACGGTTCGAATG	58	497	[41]
	int1-R	TCGTTTGTTCGCCCAGC			
Int2	*int2*-F	GGCAGACAGTTGCAAGACAA	58	247	[41]
	int2-R	AAGCGATTTTCTGCGTGTTT			
Int3	*int3*-F	CCGGTTCAGTCTTTCCTCAA	58	155	[41]
	int3-R	GAGGCGTGTACTTGCCTCAT			
Integrative and conjugative elements					
Int_{sxt}	int_{SXT}-F	GCTGGATAGGTTAAGGGCGG	58	592	[41]
	int_{SXT}-R	CTCTATGGGCACTGTCCACATTG			
Virulence genes					
stx1[a]	*stx-1*-F	CAACACTGGATGATCTCAG	55	349	[42]
	stx-1-R	CCCCCTCAACTGCTAATA			
stx2[a]	*stx-2*-F	ATCAGTCGTCACTCACTGGT	55	110	[42]
	stx-2-R	CTGCTGTCACAGTGACAAA			
invA[b]	*invA*-F	GTGAAATTATCGCCACGTTCGGGCAA	58	284	[43]
	invA-R	TCATCGCACCGTCAAAGGAACC			
spvR[b]	*spvR*-F	CAGGTTCCTTCAGTATCGCA	58	310	[43]
	spvR-R	TTTGGCCGGAAATGGTCAGT			
fimA[b]	*fimA*-F	CCTTTCTCCATCGTCCTGAA	58	85	[43]
	fimA-R	TGGTGTTATCTGCCTGACCA			
stn[b]	*stn*-F	CTTTGGTCGTAAAATAAGGCG	58	260	[43]
	stn-R	TGCCCAAAGCAGAGAGATTC			
QRDR region					
gyrA	*gyrA*-F	GCTGAAGAGCTCCTATCTGG	58	436	[44]
	gyrA-R	GGTCGGCATGACGTCCGG			
parC	*parC*-F	GTACGTGATCATGGATCGTG	58	390	[44]
	parC-R	TTCCTGCATGGTGCCGTCG			

[a] represented the gene that used for only *E. coli* isolates.
[b] represented the gene that used for only *S. enterica* isolates.

2.4. DNA preparation and PCR amplification

Preparation of genomic bacterial DNA was carried out using the whole cell boiling method [23]. A loopful of the bacterial isolate on nutrient agar (Difco) was suspended into 200 μL of sterile RNase-free water. The suspension was boiled for 10 min in a water bath and iced for 10 min, and the suspension then centrifuged at 14,000 rpm for 10 min to remove debris. The supernatant containing DNA was transferred to a sterile tube and stored at −20 °C until used as a template in the PCR assays.

The 25 μl PCR reaction mixture consisted of 12.5 μL 2 × Dream Taq PCR Master, 1 μL each primer, 1 μL template DNA, and 9.5 μL nuclease-free water. The concentration of primers, the annealing temperatures, and the sizes of the amplicons are shown in Table 2. The thermal cycling conditions were carried out at 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, the annealing temperature for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR product was analyzed by performing 1.5% agarose gel electrophoresis stained with Redsafe™ nucleic acid staining solution (Intron Biotechnology, Seongnam, Republic of Korea) for 40 min at 120 V using a horizontal electrophoresis apparatus. The gels were visualized using a UV transilluminator coupled with a digital gel imaging system.

2.5. Genotypic detection of AMR, ESBL, virulence factors, and integrons

All primers used for AMR, ESBL, virulence factors and integrons are described in Table 2. The presence of AMR genes was determined in all isolates with the corresponding resistance phenotype.

The presence of virulence genes associated with the pathogenesis of *S. enterica* (*invA, fimA, stn,* and *spvR*) and *E. coli* (*stx1* and *stx2*) were also determined. For resistance determinants, the presence of Integrative and Conjugative Elements (ICEs; Int_{sxt}) and class 1, 2, and 3 integrons (*int1, int2*, and *int3*) was determined in all isolates.

2.6. Nucleotide sequencing of ESBL and QRDR

Three *E. coli* isolates carrying bla_{CTX} and one *S. enterica* carrying both bla_{CTX} and bla_{TEM} were submitted for nucleotide sequencing. Nine isolates of resistance to ciprofloxacin were selected, including one *S. enterica isolate* and eight *E. coli* isolates to characterize the QRDR mutation, and two susceptible ciprofloxacin isolates from each of *S. enterica* and *E. coli* served as negative control strains. All bacterial isolates were examined for the presence of *gyrA* and *parC* using the primers listed in Table 2.

All PCR products were purified and sequenced (Macrogen, Inc., Seoul, Republic of Korea), with sequences analyzed using the Molecular Evolutionary Genetic Analysis (Mega) software version 11 [45]. Reference sequences were downloaded from the GenBank database available from the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov). The accession numbers for the various genes were as follows: *gyr*A: OQ268120, OQ268121, OQ268122, OQ268123, OQ268124, OQ268125, OQ268126, OQ268127, OQ268128; parC: OQ268129, OQ268130, OQ268131, OQ268132, OQ268133, OQ268134, OQ268135, OQ268136, OQ268137; blaCTX-M-55: OQ281750, OQ281751, OQ281752, OQ281753; blaCTX-M-15: OQ281754; blaTEM-1: OQ281755.

2.7. Statistical analysis

Descriptive statistics were used to describe the prevalence of AMR, MDR, ESBL production, and virulence factors for the group of *S. enterica* and *E. coli* isolates. McNemar's chi-square test was used to examine the association between the AMR phenotype and the genotype. The median and range of MICs between susceptible and resistance bacteria were also compared. Logistic regression was used to determine the association between the odds of tetracycline resistance and the various virulence factors, non-tetracycline resistance, and their genetic determinants. Odds ratio (OR) > 1 is considered a positive relationship, while OR < 1 is a negative relationship. Stepwise forward selection and backward elimination were used to select the final statistical model. The two-sided hypotheses test was used based on a P - value < 0.05. All statistical analyses were performed with SPSS version 22 (IBM Company, Chicago, USA).

Table 3
Phenotypic resistance of *S. enterica* ($n = 59$) and *E. coli* ($n = 392$) isolated from produce commodities from supermarkets and open-air fresh markets throughout Bangkok, Thailand.

Antimicrobials	No. of resistance isolates (%)			
	Supermarket		Fresh market	Total
	Conventional	Organic	Conventional	
S. enterica	***n* = 19**	***n* = 3**	***n* = 37**	***n* = 59**
Ampicillin	0 (0.0)	1 (33.3)	3 (8.1)	4 (6.8)
Tetracycline	0 (0.0)	1 (33.3)	6 (16.2)	7 (11.9)
Trimethoprim	0 (0.0)	1 (33.3)	1 (2.7)	2 (3.4)
Sulfamethoxazole	0 (0.0)	1 (33.3)	2 (5.4)	3 (5.1)
Streptomycin	0 (0.0)	1 (33.3)	4 (10.8)	5 (8.5)
Ciprofloxacin	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
Chloramphenicol	0 (0.0)	1 (33.3)	1 (2.7)	2 (3.4)
Gentamicin	0 (0.0)	1 (33.3)	2 (5.4)	2 (3.4)
Colistin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime	0 (0.0)	2 (66.7)	2 (5.4)	4 (6.8)
Cefotaxime	0 (0.0)	1 (33.3)	2 (5.4)	3 (5.1)
Cefpodoxime	0 (0.0)	1 (33.3)	2 (5.4)	3 (5.1)
ESBL producer	0 (0.0)	1 (33.3)	2 (5.4)	3 (5.1)
MDR	0 (0.0)	1 (33.3)	3 (8.1)	4 (6.8)
E. coli	***n* = 115**	***n* = 98**	***n* = 179**	***n* = 392**
Ampicillin	25 (21.7)	22 (22.5)	37 (20.7)	84 (21.4)
Tetracycline	29 (25.2)	21 (21.4)	38 (21.2)	88 (22.5)
Trimethoprim	15 (13.0)	11 (11.2)	15 (8.4)	41 (10.5)
Sulfamethoxazole	12 (10.4)	13 (13.3)	20 (11.2)	45 (11.5)
Streptomycin	12 (10.4)	7 (7.1)	18 (10.1)	37 (9.4)
Ciprofloxacin	4 (3.5)	0 (0.0)	4 (2.2)	8 (2.0)
Chloramphenicol	6 (5.2)	9 (9.2)	8 (4.5)	23 (5.8)
Gentamicin	1 (0.8)	0 (0.0)	4 (2.2)	5 (1.3)
Colistin	2 (1.7)	0 (0.0)	0 (0.0)	2 (0.5)
Imipenem	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)
Meropenem	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime	2 (1.7)	1 (1.0)	1 (1.1)	4 (1.0)
Cefotaxime	2 (1.7)	2 (2.0)	2 (1.1)	6 (1.5)
Cefpodoxime	2 (1.7)	2 (2.0)	2 (1.1)	6 (1.5)
ESBL producer	2 (1.7)	1 (1.0)	1 (0.6)	4 (1.0)
MDR	19 (16.5)	14 (14.3)	27 (15.1)	60 (15.3)

MDR – multidrug resistant.

3. Results

3.1. Prevalence and patterns of AMR for isolates of S. enterica and E. coli

Fifteen percent of all isolates of *S. enterica,* ($n = 9/59$) were resistant to at least one antimicrobial, while the prevalence of MDR was 6.8% ($n = 4/59$). The highest prevalence of AMR in *S. enterica* was to tetracycline (11.9%, $n = 7/59$), followed by streptomycin (8.5%, $n = 5/59$), ampicillin (6.8%, $n = 4/59$), ceftazidime (6.8%, $n = 4/59$), and sulfamethoxazole (5.1%, $n = 3/59$) (Table 3). Resistance to colistin, imipenem, and meropenem was not observed among these isolates. The bacteria isolated from cucumbers exhibited the highest prevalence of AMR, which was against tetracycline (33.3%, $n = 1/3$), but the low sample size of the isolates for this commodity makes this value somewhat unreliable (Fig. 1A). Furthermore, *S. enterica* isolated from cucumber and coriander were more frequently AMR compared to isolates from other products, while no AMR isolates were obtained from spring onion and lettuce (Fig. 1A). It is interesting that none of the *S. enterica* isolated from conventional produce from all 19 of the sampled supermarkets exhibited phenotypic AMR; in contrast, one of the three isolates of *S. enterica* from organic produce from supermarkets exhibited MDR (Table 3).

The median MIC for the nine *S. enterica* isolates exhibiting AMR was 3 times higher than the clinical breakpoint for all antimicrobials tested, except for colistin since all isolates were susceptible to this antibiotic (Fig. 2A). Furthermore, the median MICs for trimethoprim, sulfamethoxazole, chloramphenicol, and gentamicin exceeded the upper MIC detection limit for this study. For example, the median MIC for sulfamethoxazole resistance was higher than 4096 μ g/mL, which is at least four-fold higher than its clinical breakpoint of 512 μ g/mL. Six AMR patterns were observed among these AMR *S. enterica* isolates, with the most common pattern being TET (6.8%, $n = 4/59$), followed by 1.7% ($n = 1/59$) of each pattern of AMP-CHL-CIP-GEN-SMZ-STR-TET-TMP, AMP-CHL-GEN-SMZ-STR-TET-TMP, AMP-GEN-STR, AMP-SMZ-STR-TET and STR (Table 4).

One hundred and three out of 392 *E. coli* isolates (26.3%) exhibited phenotypic AMR to at least one or more antimicrobials, with about half (15.3%, $n = 60/392$) of these isolates exhibiting MDR. The highest prevalence of AMR was to tetracycline (22.5%, $n = 88/392$), followed by ampicillin (21.4%, $n = 84/392$), sulfamethoxazole (11.5%, $n = 45/392$), trimethoprim (10.5%, $n = 41/392$), and streptomycin (9.4%, $n = 37/392$) (Table 3, Fig. 1B). Resistance to tetracycline was highest in isolates from tomatoes (41.4%, $n = 12/29$), followed by spring onions (32.3%, $n = 20/62$) and cabbage (29.4%, $n = 10/34$). Similarly, a high prevalence of ampicillin resistance among *E. coli* was observed for isolates from tomatoes (34.5%), spring onions (33.9%) and cabbage (26.5%). *E. coli* isolated

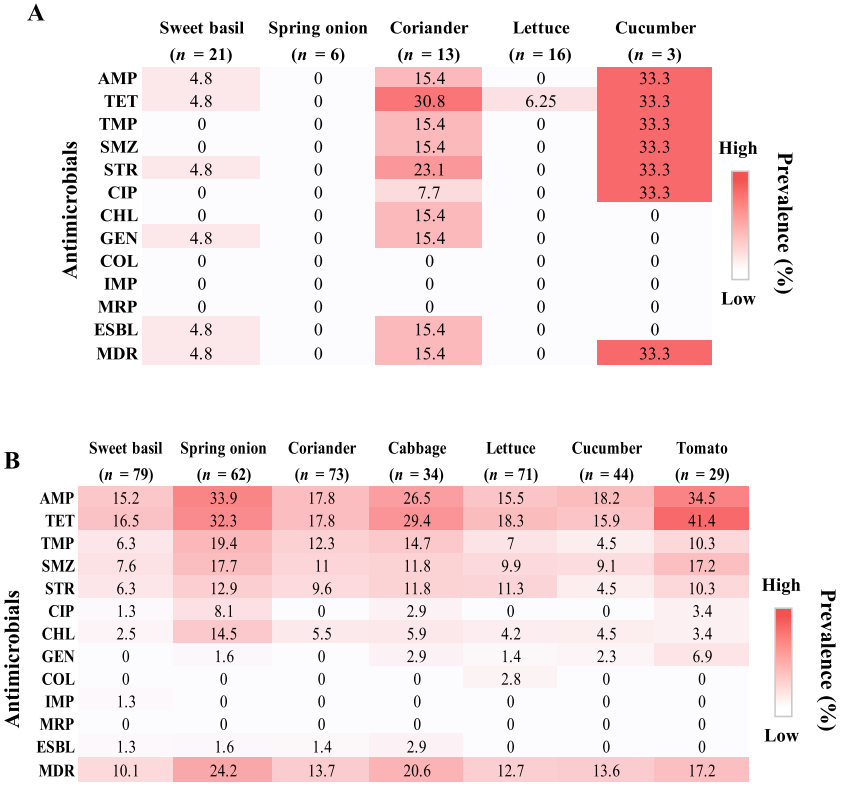

Fig. 1. The distribution of phenotypic resistance of (A) *S. enterica* ($n = 59$) and (B) *E. coli* ($n = 392$) isolated from different produce commodities common to Thai cuisine. Antimicrobials tested were ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), Imipenem (IMP), meropenem (MRP), sulfamethoxazole (SMZ), streptomycin (STR), tetracycline (TET), and trimethoprim (TMP). ESBL – extended-spectrum β-lactamase; MDR – multidrug resistant. Percentages within parentheses are within-column proportions (for example, cucumbers provided three *S. enterica* isolates, among which 33.3% or 1/3 exhibited ampicillin resistance).

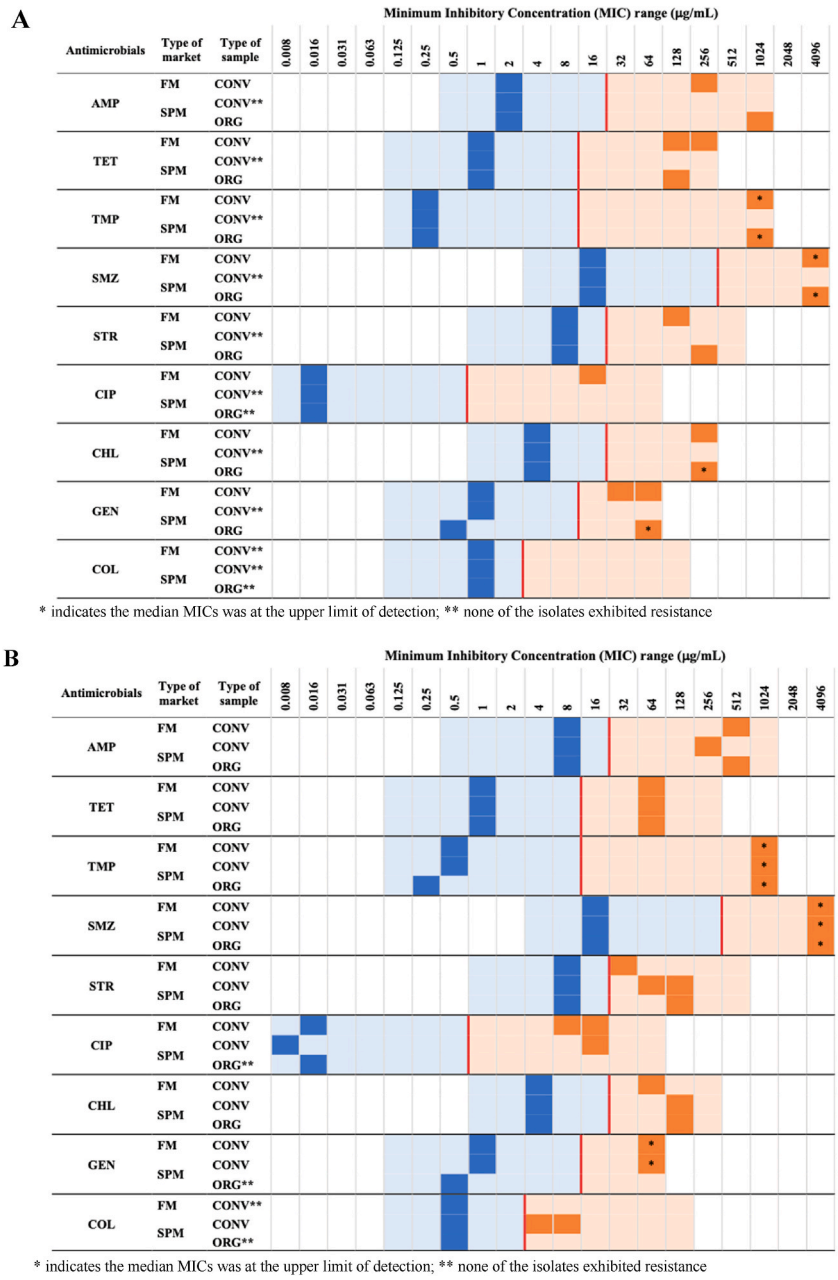

Fig. 2. The range of minimum inhibitory concentrations (MICs) for the group of (A) *S. enterica* ($n = 59$) and (B) *E. coli* ($n = 392$) isolates. The concentration ranges of each antimicrobial agent used for the antimicrobial susceptibility test are shown as light blue (susceptible range) and light orange (resistance range) with a clinical breakpoint (red line) for resistance. The median MICs are indicated at the susceptible point (blue) and resistance point (orange). AMP – ampicillin; CHL – chloramphenicol; CIP – ciprofloxacin; COL – colistin; GEN – gentamicin; SMZ – sulfamethoxazole; STR – streptomycin; TET – tetracycline; TMP – trimethoprim; FM – fresh market; SPM – supermarket; CONV – conventional produce; ORG – organic produce.

from spring onions exhibited the highest prevalence of MDR (24.2%), followed by isolates from cabbage (24.2%) (Fig. 1B). The median MICs of trimethoprim, sulfamethoxazole, and gentamicin were above the detection limit (Fig. 2B). The prevalence of AMR for *E. coli* isolated from supermarket produce reportedly grown under conventional methods compared organic conditions were not significantly different ($P > 0.05$).

In this study, 38 *E. coli* AMR patterns were observed, with the predominant resistance patterns being AMP-TET (5.4%, $n = 21/392$), followed by AMP-STR-TET (2.8%, $n = 11/392$), and TET (1.8%, $n = 7/392$) (Table 5).

Table 4
Resistance patterns of *S. enterica* ($n = 59$) isolated from produce commodities sold in supermarkets and open-air fresh markets from throughout Bangkok, Thailand.

AMR pattern	No. (%) of isolates with AMR pattern			
	Supermarket		Fresh market	Total ($n = 59$)>
	Conventional ($n = 19$)>	Organic ($n = 3$)>	Conventional ($n = 37$)>	
AMP-CHL-CIP-GEN-SMZ-STR-TET-TMP	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
AMP-CHL-GEN-SMZ-STR-TET-TMP	0 (0.0)	1 (33.3)	0 (0.0)	1 (1.7)
AMP-GEN-STR	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
AMP-SMZ-STR-TET	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
STR	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
TET	0 (0.0)	0 (0.0)	4 10.8)	4 (6.8)

AMP – ampicillin, CHL – chloramphenicol, CIP – ciprofloxacin, GEN – gentamicin, SMZ – sulfamethoxazole, STR – streptomycin, TET – tetracycline, TMP – trimethoprim.

Table 5
Resistance patterns of *E. coli* ($n = 392$) isolated from produce commodities sold in supermarkets and open-air fresh markets from throughout Bangkok, Thailand.

AMR pattern	No. (%) of isolates with AMR pattern			
	Supermarket		Fresh market	Total ($n = 392$)>
	Conventional ($n = 115$)>	Organic ($n = 98$)>	Conventional ($n = 179$)>	
AMP	2 (1.7)	0 (0.0)	0 (0.0)	2 (0.5)
AMP-CHL-CIP-GEN-SMZ-STR-TET-TMP	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
AMP-CHL-CIP-SMZ-TET-TMP	1 (0.7)	0 (0.0)	3 (1.7)	4 (1.0)
AMP-CHL-COL-SMZ-STR-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
AMP-CHL-GEN-SMZ-STR-TET	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
AMP-CHL-GEN-SMZ-TET-TMP	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
AMP-CHL-SMZ-STR-TET	1 (0.7)	1 (1.0)	0 (0.0)	2 (0.5)
AMP-CHL-SMZ-STR-TET-TMP	1 (0.7)	4 (4.1)	1 (0.6)	6 (1.5)
AMP-CHL-SMZ-TET	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)
AMP-CHL-SMZ-TET-TMP	0 (0.0)	2 (2.0)	0 (0.0)	2 (0.5)
AMP-CHL-SMZ-TMP	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)
AMP-CHL-TET-TMP	1 (0.7)	0 (0.0)	1 (0.6)	2 (0.5)
AMP-CIP-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
AMP-GEN	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
AMP-GEN-SMZ-STR-TET	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
AMP-IMP	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)
AMP-SMZ	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
AMP-SMZ-STR-TET	1 (0.7)	0 (0.0)	2 (1.1)	3 (0.8)
AMP-SMZ-STR-TET-TMP	1 (0.7)	1 (1.0)	3 (1.7)	5 (1.3)
AMP-SMZ-TET	1 (0.7)	1 (1.0)	1 (0.6)	3 (0.8)
AMP-SMZ-TET-TMP	1 (0.7)	2 (2.0)	3 (1.7)	6 (1.5)
AMP-SMZ-TMP	0 (0.0)	0 (0.0)	2 (1.1)	2 (0.5)
AMP-STR-TET	3 (2.6)	1 (1.0)	7 (3.9)	11 (2.8)
AMP-STR-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
AMP-TET	5 (4.3)	7 (7.1)	9 (5.0)	21 (5.4)
AMP-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
AMP-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
CHL-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
CIP	2 (1.7)	0 (0.0)	0 (0.0)	2 (0.5)
COL	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
SMZ-STR-TET	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
SMZ-TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
SMZ-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
STR	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
STR-TET	2 (1.7)	0 (0.0)	1 (0.6)	3 (0.8)
TET	3 (2.6)	1 (1.0)	3 (1.7)	7 (1.8)
TET-TMP	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)
TMP	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)

AMP – ampicillin, CHL – chloramphenicol, CIP – ciprofloxacin, COL – colistin, GEN – gentamicin, IMP – imipenem, MRP – meropenem, SMZ – sulfamethoxazole, STR – streptomycin, TET – tetracycline, TMP – trimethoprim.

3.2. Genetic analysis of AMR in S. enterica and E. coli isolates

Among all resistance genes that were analyzed, *qnrS* (11.9%) was the most common among *S. enterica* isolates, followed by *tetB* (6.8%), *tetA* (5.1%), *strA* (5.1%), *strB* (5.1%), and *qnrB* (5.1%). The least common AMR gene found in *S. enterica* was *dfrA12*, *sul2*, *sul3*, *cmlA*, bla_{TEM}, and *qacE* (Table 6). Interestingly, a single *S. enterica* isolate from sweet basil from a supermarket carried bla_{TEM}, *tetB*, *strA,* and *strB* (data not shown). Among supermarket organic vegetable isolates ($n = 3$), the most predominant resistance gene was *qnrS* (66.7%), found in spring onions and coriander, followed by single detection (1/3) of *tetA*, *sul2*, *strA*, *strB*, and *floR* (data not shown). For conventional vegetables from fresh markets, the most frequent AMR gene was the *qnrS* (13.5%, $n = 5/37$), which was found in sweet basil, followed by *qnrB* (8.1%), *tetB* (8.1%), *tetA* (5.4%), and *aadA* (5.4%) (data not shown).

Among *E. coli* isolates, the predominant resistance genes were bla_{TEM} (20.2%), *tetA* (19.1%), and *qnrS* (14.8%) (Table 7). In *E. coli* isolates from conventional vegetables collected from supermarkets ($n = 115$), the prevalence of resistance genes for *tetA,* bla_{TEM}, and *qnrS* was 20.0%, 18.3%, and 15.7%, respectively (Table 7). For supermarket organic vegetable isolates ($n = 98$), the predominant AMR genes were bla_{TEM} (23.5%), *tetA* (22.4%) and *qnrS* (16.3%). For conventional vegetables from fresh markets ($n = 179$), bla_{TEM} (19.6%) was the most common among the isolates, followed by *tetA* (16.8%), *qnrS* (13.4%), *sul2* (6.1%), and *sul3* (6.1%) (Table 7).

Overall, the prevalence of AMR genes detected in these produce commodities did not differ significantly ($P > 0.05$) between reported conventional and organic cultivation practices for the *S. enterica* and *E. coli* isolates.

A single *S. enterica* and eight *E. coli* isolated from conventional cultivation practices that were resistant to ciprofloxacin had *gyrA* and *parC* mutations. *S. enterica* ($n = 1$) isolated from conventional coriander with co-occurrence *qnrS* was found to have the double-point mutation in *gyrA* (Ser83Phe and Asp87Gly) and a single-point mutation in *parC* (Ser80Arg), with MIC of 16 μ g/mL. Among the eight *E. coli* isolates, the frequency of resistance to ciprofloxacin was detected mainly in spring onions ($n = 5/8$) (Table 8). For the *gyrA* gene, all *E. coli* isolates were found to have a double point mutation in Ser83Leu and Asp87Asn with and without the presence of the *qnrS* gene. Seven out of eight *E. coli* isolates had a *parC* mutation in Ser80Ile ($n = 4$), with MICs between 4 and 16 μ g/mL and double point mutation in Ser80Ile and Glu84Gly ($n = 3$), with MICs between 16 and 32 μ g/mL. An *E. coli* isolate with a low MIC (1 μ g/mL) did not exhibit the mutation of *parC*.

3.3. ESBL production in S. enterica and E. coli isolates

A small proportion of the *S. enterica* (5.1%, $n = 3/59$) isolates exhibited ESBL production (Table 3), which were only found in coriander (15.4%, $n = 2/13$) and sweet basil (4.8%, $n = 1/21$) (Fig. 1A). Among the three ESBL positive isolates, an organic produce (coriander) collected in the supermarket was *S. enterica* serovar Afula (data not shown), and the other two conventional vegetables (coriander and sweet basil) from the fresh market were *S. enterica* serovar Schwarzengrunt and Uganda (data not shown). All ESBL

Table 6
Genotypic characteristics of *S. enterica* ($n = 59$) isolated from produce commodities sold in supermarkets and open-air fresh markets from throughout Bangkok, Thailand.

Genes	No. (%) of isolates with specified gene			
	Supermarket		Fresh market	Total ($n = 59$)>
	Conventional ($n = 19$)>	Organic ($n = 3$)>	Conventional ($n = 37$)>	
AMR genes				
bla_{TEM}	1 (5.3)	0 (0.0)	1 (2.7)	2 (3.4)
tetA	0 (0.0)	1 (33.3)	2 (5.4)	3 (5.1)
tetB	1 (5.3)	0 (0.0)	3 (8.1)	4 (6.8)
dfrA12	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
sul2	0 (0.0)	1 (33.3)	0 (0.0)	1 (1.7)
sul3	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
strA	1 (5.3)	1 (33.3)	1 (2.7)	3 (5.1)
strB	1 (5.3)	1 (33.3)	1 (2.7)	3 (5.1)
aadA	0 (0.0)	0 (0.0)	2 (5.4)	2 (3.4)
qnrB	0 (0.0)	0 (0.0)	3 (8.1)	3 (5.1)
qnrS	0 (0.0)	2 (66.7)	5 (13.5)	7 (11.9)
cmlA	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
floR	0 (0.0)	1 (33.3)	1 (2.7)	2 (3.4)
$bla_{CTX-M-55}$	0 (0.0)	1 (33.3)	1 (2.7)	2 (3.4)
bla_{TEM-1}	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
qacE	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)
Integrons				
int1	0 (0.0)	0 (0.0)	2 (5.4)	2 (3.4)
Virulence genes				
invA	19 (100.0)	3 (100.0)	36 (97.3)	58 (98.3)
spvR	19 (100.0)	3 (100.0)	37 (100.0)	59 (100.0)
fimA	19 (100.0)	3 (100.0)	36 (97.3)	58 (98.3)
stn	0 (0.0)	0 (0.0)	1 (2.7)	1 (1.7)

Note: only positive genes were shown in this table.

Table 7
Genotypic characteristics of *E. coli* ($n = 392$) isolated from produce commodities sold in supermarkets and open-air fresh markets from throughout Bangkok, Thailand.

Genes	No. (%) of isolates with specified gene			
	Supermarket		Fresh market	Total ($n = 392$)>
	Conventional ($n = 115$)	Organic ($n = 98$)	Conventional ($n = 179$)>	
AMR genes				
bla$_{TEM}$	21 (18.3)	23 (23.5)	35 (19.6)	79 (20.2)
bla$_{PSE}$	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.3)
tetA	23 (20.0)	22 (22.4)	30 (16.8)	75 (19.1)
tetB	3 (2.6)	0 (0.0)	2 (1.1)	5 (1.3)
tetW	2 (1.7)	5 (5.1)	3 (1.7)	10 (2.6)
dfrA1	2 (1.7)	0 (0.0)	1 (0.6)	3 (0.8)
dfrA12	3 (2.6)	7 (7.1)	7 (3.9)	17 (4.3)
dfrA14	6 (5.2)	4 (4.1)	5 (2.8)	15 (3.8)
sul1	0 (0.0)	1 (1.0)	5 (2.8)	6 (1.5)
sul2	7 (6.1)	5 (5.1)	11 (6.1)	23 (5.9)
sul3	3 (2.6)	6 (6.1)	11 (6.1)	20 (5.1)
strA	11 (9.6)	6 (6.1)	9 (5.0)	26 (6.6)
strB	10 (8.7)	6 (6.1)	9 (5.0)	25 (6.4)
aadA	5 (4.3)	7 (7.1)	8 (4.5)	20 (5.1)
qnrA	0 (0.0)	1 (1.0)	0 (0.0)	1 (0.3)
qnrB	0 (0.0)	2 (2.0)	0 (0.0)	2 (0.5)
qnrS	18 (15.7)	16 (16.3)	24 (13.4)	58 (14.8)
cmlA	3 (2.6)	7 (7.1)	8 (4.5)	18 (4.6)
floR	6 (5.2)	9 (9.2)	6 (3.4)	21 (5.4)
aadB	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.3)
mcr-1	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.3)
bla$_{CTX-M-15}$	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.3)
bla$_{CTX-M-55}$	1 (0.9)	0 (0.0)	1 (0.6)	2 (0.5)
qacE	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.3)
Integrons				
int1	11 (9.6)	7 (7.1)	9 (5.0)	27 (6.9)

Note: only positive genes were shown in this table.

Table 8
Ciprofloxacin resistant *E. coli* ($n = 8$) and *S. enterica* ($n = 1$) isolates from conventional produce cultivation with PMQR genes and *gyrA* and *parC* mutations in the QRDR.

ID	Source	Vegetable	MIC (μg/mL)	PMQR	QRDR			
					gyrA mutation		*parC* mutation	
EC035	FM	Spring onion	1	*qnrS*	Ser83Leu	–	–	–
EC047	FM	Spring onion	4	–	Ser83Leu	Asp87Asn	Ser80Ile	–
EC078	FM	Tomato	16	–	Ser83Leu	Asp87Asn	Ser80Ile	–
EC106	FM	Spring onion	32	*qnrS*	Ser83Leu	Asp87Asn	Ser80Ile	Glu84Gly
EC179	SPM	Sweet basil	16	–	Ser83Leu	Asp87Asn	Ser80Ile	–
EC239	SPM	Spring onion	16	–	Ser83Leu	Asp87Asn	Ser80Ile	Glu84Gly
EC247	SPM	Spring onion	4	*qnrS*	Ser83Leu	–	Ser80Ile	–
EC377	SPM	Cabbage	32	*qnrS*	Ser83Leu	Asp87Asn	Ser80Ile	Glu84Gly
SE01	FM	Coriander	16	*qnrS*	Ser83Phe	Asp87Asn	Ser80Arg	–

EC – *E. coli*, SE – *S. enterica*, FM – fresh market, SPM – supermarket, MIC – minimum inhibitory concentration, PMQR – plasmid-mediated quinolone resistance, QRDR – quinolone-resistance determining region.

producing isolates were MDR, in which a serovar Afula harbored *bla*$_{CTX-M-55}$ and a serovar Uganda had both *bla*$_{TEM-1}$ and *bla*$_{CTX-M-55}$, while a serovar Schwarzengrunt contained none of these genes.

Similar to *S. enterica*, a small proportion of *E. coli* isolates (1.0%, $n = 4/392$) exhibited ESBL production, with one isolate from organically-grown commodities (1.0%, $n = 1/98$, sweet basil), two isolates from conventionally-grown commodities (1.7%, $n = 2/115$, spring onion and coriander) from a supermarket, and one conventionally-grown cabbage (0.6%, $n = 1/179$) from a fresh market (Fig. 1B). Two *E. coli* isolated from sweet basil and cabbage that produced ESBL were MDR. Based on the nucleotide sequence, the two ESBL-producing *E. coli* isolates of spring onion and coriander collected from supermarkets carried *bla*$_{CTX-M-55}$ and *bla*$_{CTX-M-15}$, respectively. Furthermore, an *E. coli* isolate from a fresh market tomato, which did not produce ESBL, contained *bla*$_{CTX-M-55}$ (Table 7).

3.4. Occurrence of virulence genes, ICEs, and class 1, 2, and 3 integrons

All *S. enterica* isolates harbored *spvR* (100.0%), followed by *invA* (98.3%) and *fimA* (98.3%), while *stn* (1.7%) was the least detected

gene (Table 6). None of the *E. coli* isolates had detectable *stx1* and *stx2* (Table 7). The presence of *int1* was observed in two isolates of *S. enterica* (3.4%) and 27 isolates of *E. coli* (6.9%) (Tables 6 and 7). None of these *S. enterica* and isolates *E. coli* contained ICEs (int_{SXT}) and integrons (*int2* and *int3*).

3.5. Associations between AMR phenotype and genotype

The association between the AMR phenotype and genotype, virulence factors, the production of ESBL, and resistance determinants was examined separately among *S. enterica* and *E. coli* isolates. The dependent variable (outcome) was the presence or absence of tetracycline resistance based on its high prevalence among these isolates of *S. enterica* and *E. coli*. The odds of resistance to tetracycline among isolates of *S. enterica* were positively associated with the co-occurrence of ampicillin resistance (OR = 5.01, $P = 0.001$) and the presence of *tetB* (OR = 5.01, $P = 0.001$) (Table 9). The odds of resistance to tetracycline among isolates of *E. coli* were positively associated with ampicillin resistance (OR = 3.64, $P < 0.0001$), streptomycin resistance (OR = 5.45, $P < 0.0001$), and the presence of *tetA* (OR = 6.75, $P < 0.0001$) (Table 9).

4. Discussion

4.1. AMR and its determinants, virulence factors, and semi-quantitative analysis

This study has demonstrated the occurrence of virulence factors, AMR, ESBL production, and resistance determinants among *S. enterica* and *E. coli* isolated from fresh vegetables and fruits from retail markets throughout Bangkok, Thailand. Approximately 50% of resistant *S. enterica* and *E. coli* isolates exhibited MDR. While previous study by Srisamran et al. [19] indicated a higher occurrence of *E. coli* contamination in fresh market samples compared to those from supermarkets, a relatively comparable prevalence of AMR and MDR *E. coli* was observed in samples from both types of markets. With respect to *S. enterica* isolated from these two retail venues, the prevalence of AMR was over 2-times higher in isolates from fresh market samples (21.6%) compared to isolates from supermarket samples (9.1%), with the prevalence of MDR similarly higher in fresh market samples (8.1%) compared to isolates from supermarket samples (4.5%). This suggests that the levels of AMR and MDR in *E. coli* were not significantly affected by the source of fresh fruits and vegetables. Nevertheless, the phenotypic AMR in *Salmonella* isolated from fresh markets was approximately twice as high as that found in supermarkets. Multiple processes of AMR contamination can operate at either the production field level (e.g., runoff from agricultural lands, irrigation water supplies contaminated with AMR bacteria from domestic or municipal sewage, use of unprocessed livestock manure as soil amendment), during processing and distribution (e.g., rodent fecal contamination of storage bins, inadequate personal hygiene of packers), or during retail display (e.g., fecal contamination of display cases, excessive handling by consumers) [5, 46–48]. Given that the arithmetic mean concentration of *E. coli* eluted from the surface of fresh fruits and vegetables from supermarkets and fresh markets grown under conventional conditions was 5.3×10^5 and 1.1×10^3 MPN/g [19], combined with the observation that 15–25% of these *E. coli* isolates were either AMR or MDR, there is ample opportunity for human exposure and potential oral consumption of these resistant bacteria from Bangkok's retail distribution of fresh fruits and vegetables. Exposure to AMR *S. enterica*, especially with isolates that harbor one more virulence genes, further heightens the public health and food safety concern when consuming produce items either raw or minimally washed, as can often occur in popular dishes of Thai cuisine.

In this study, the most common resistance phenotype exhibited by *S. enterica* was tetracycline (11.9%), with a median MIC between 128 and 256 μ g/mL. This relatively high MIC value is at least three times higher than the clinical breakpoint of 16 μg/mL for tetracycline, indicating that infection by these AMR bacteria may cause a failure of tetracycline treatment. However, a low prevalence of resistance (<10%) was observed for many of the antimicrobials tested in this study, including streptomycin, ampicillin, sulfamethoxazole, trimethoprim, chloramphenicol, gentamicin, and ciprofloxacin. During the past decade, MDR salmonellosis has been considered endemic in many developing countries, especially South-central and Southeast Asia [49]. In the same study, resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole has been reported to lead to the use of FQs (e.g., ciprofloxacin) as an

Table 9
Multivariate logistic regression of factors associated with tetracycline resistance in *S. enterica* ($n = 59$) and *E. coli* ($n = 392$) isolates.

Predictor	Odds ratio	Std. Err.[a]	95% C.I.[b]	*P* - value
S. enterica				
Ampicillin resistance	5.01	1.53	2.00–8.02	0.001
tetB	5.01	1.53	2.00–8.02	0.001
Constant	−3.9	1.01	−5.89 – (−1.93)	<0.0001
AIC[c]	24.84			
E. coli				
Ampicillin resistance	3.64	0.83	2.01–5.27	<0.0001
Streptomycin resistance	5.45	1.30	2.90–8.00	<0.0001
tetA	6.75	1.17	4.45–9.04	<0.0001
Constant	−4.65	0.58	−5.79 – (−3.51)	<0.0001
AIC[c]	65.32			

[a] Std. Err.: Standard Error.
[b] C.I.: Confidence Interval.
[c] AIC: Akaike Information Criteria.

alternative antimicrobial for the treatment of human salmonellosis. In this study, only 1.7% of *S. enterica* isolates were resistant to ciprofloxacin, which was isolated from conventional coriander collected from a fresh market. Although there was a low prevalence of resistance to ciprofloxacin, the median MIC was 16 μ g/mL, which was four times higher than the clinical breakpoint. Similarly, the MICs of *Salmonella* isolates resistant to chloramphenicol, ampicillin, trimethoprim, and sulfamethoxazole were 3, 3–5, 7, and 4 times higher than the susceptible cutoff point. This finding indicates that human and animal infection with these AMR *S. enterica* could pose a serious health threat and lead to failure of treatment due to the limited choices of effective antimicrobials.

In this study, it was observed that *E. coli* isolates showed significant resistance to tetracycline (22.5%) and ampicillin (21.4%), with median MIC values 3 times higher for tetracycline and 4–5 times higher for ampicillin compared to the cutoff values. This finding was consistent with previous studies in fresh produce whereby the majority of AMR was against tetracycline and ampicillin [48,50]. Resistance to tetracycline and ampicillin has been commonly reported in livestock and in environmental samples [46,51,52]. Therefore, it is not surprising to find these resistant bacteria circulating in the environment and capable of contaminating fresh fruits and vegetables. Furthermore, these two antimicrobials are classified as Highly Important Antimicrobials (HIA) by the World Health Organization (WHO) and Veterinary Critically Important Antimicrobial Agents (VCIA) by the World Organization for Animal Health (OIE) [53,54], implying the health threats posed by AMR for One Health (human, animal, and environmental health).

It should be noted that chloramphenicol is one of the prohibited antimicrobials in food-producing animals according to the regulations of the Ministry of Agriculture and Cooperatives of Thailand [55]. However, this study found a prevalence of chloramphenicol resistance of 3.4% in *Salmonella* and 5.8% in *E. coli*, with median MIC values between 64 and 256 μ g/mL, which was higher than the clinical breakpoint, and all chloramphenicol resistant isolates were MDR. Furthermore, the *cmlA* and *floR* genes that encode for chloramphenicol resistance were detected in more than half of all resistant isolates (56% in *Salmonella* and 84% in *E. coli*), which also coexist with *int1* gene (56%). The cassette-borne *cmlA* group is commonly associated with MDR integrons or transposons in a range of enteric genera. Additionally, the independent acquisition of mobile elements carrying *cat*, *cmlA*, *floR*, or *cfr* genes can result in the simultaneous presence of multiple types of phenicol resistance genes within the same bacteria, even in cases where the use of chloramphenicol in food-producing animals is limited [56,57].

4.2. ESBL-producing isolates and their genotype

ESBL-producing bacteria are of increasing public health concern, with these bacteria among the most important causes of nosocomial and community-acquired infections [58]. ESBL genes can spread through horizontal gene transfer, which may harbor additional AMR genes [46]. In contrast to prior research, the occurrence of ESBL-producing bacteria in fresh fruits and vegetables was lower in this study [2,17,59]. Furthermore, $bla_{\text{CTX-M}}$ was the only ESBL gene detected in this study and $bla_{\text{CTX-M-55}}$ ($n = 4/7$) was the most common CTX-M group 1. CTX-*M*-55 has been increasingly observed from different isolates from humans, livestock and the environment in many Asian countries, including Thailand [52,60,61]. CTX-*M*-55 is a derivative of CTX-*M*-15, where CTX-*M*-55 has a single amino acid substitution (Ala77Val) that causes increased activity against ceftazidime [62]. Furthermore, in this study, one of the ESBL producing *S. enterica* isolates harbored $bla_{\text{CTX-M-55}}$ that co-occurred with $bla_{\text{TEM-1}}$. More importantly, most of the isolates with $bla_{\text{CTX-M-55}}$ were MDR ($n = 3/4$). A *S. enterica* isolate from conventional sweet basil in a fresh market containing $bla_{\text{CTX-M-55}}$ also contained TEM1 and *int1*, which was similar with a previous study reporting CTX-*M*-55 isolated from vegetables in northern Thailand [18]. In general, this study indicated that vegetables may be a source of human exposure to bacteria producing ESBL. The origins of ESBL-producing bacterial isolates are likely due to processes such as contamination from organic manure commonly used in agriculture, fecal contamination through irrigation water, agricultural runoff, or dispersal of human sewage [11,63]. Due to the complexity of environmental dissemination for ESBL-producing bacteria, further investigation by examining potential sources of contamination, such as water, soil, contact surfaces, and animals, and using molecular techniques to track dissemination of these bacteria should be performed using a One Health approach.

4.3. QRDR mutations

Quinolone resistance is commonly mediated by mutations in the QRDR of *gyrA* and *parC*. In this study, six out of eight ciprofloxacin-resistant *E. coli* isolates carried a double mutation of Ser83Leu and Asp87Asn in the *gyrA* mutation with a high MIC level, while two isolates carried a single Ser83Leu mutation in *gyrA* which had a lower MIC level than a double mutation of *gyrA*. The isolate resistant to ciprofloxacin at 1 μ g/mL did not carry any mutation in *parC*, but a single Ser80Ile *parC* mutation was resistant at 4–16 μ g/mL and a double mutation of Ser80Ile and Glu84Gly in *parC* was found in isolates resistant at 16–32 μ g/mL. This implies that a single mutation in *gyrA* can generate resistance to quinolones, but additional mutations in *gyrA* and/or *parC* are needed for high-level resistance to ciprofloxacin, which is in agreement with a previous work [52].

4.4. int1 gene with MDR phenotype

The class 1 integron (*int1*) is one of the most common mobile genetic elements that can transfer the resistance genotype to its variable regions or between bacterial species. This element has been suggested to be an indicator of AMR monitoring for the occurrence and removal efficiency of AMR genes in wastewater due to its positive association with the resistance genes. This is because *int1* was strongly correlated with human pathogens and a large number of resistance genes [64]. Although the isolates in this collection harbored *int1* with low prevalence, greater than 80% and 95% of *int1* positive isolates were MDR. Our data suggests that *int1* may be critical for monitoring AMR in the environment, especially for the MDR phenotype. This gene should be added as a target resistance

determinant for observation of MDR bacteria under the One Health paradigm.

4.5. Associations between AMR phenotype and genotype

This study addressed the association between the AMR phenotype and genotype based on logistic regression models. The odds of tetracycline resistance for these *S. enterica* isolates were five-times higher if the isolate was also resistant to ampicillin (OR = 5.0) or carried the *tetB* gene (OR = 5.0). A similar association was observed in *E. coli*; the odds of tetracycline resistance was 6.8-times higher if the isolate carried the *tetA* gene. In addition, the odds for tetracycline resistance was 3.6-times and 5.5-times higher for isolates of *E. coli* that were also resistant to ampicillin or streptomycin, respectively. This finding indicates that there was close association with or mechanisms of co-selection for resistance to tetracycline, ampicillin, and streptomycin. This co-occurrence of resistance may be the result of mobile genetic elements such as integrons and plasmids, which are important routes of AMR genomic transfer between bacteria. Associations of genes that encode resistance against several important antimicrobial classes, including aminoglycosides, β-lactams, phenicols, quinolones, tetracyclines and sulfonamides, has been previously consistently reported.

5. Conclusion

This study is the first report on semi-quantitative analysis of phenotypic and genotypic AMR on the surface of raw vegetables and fruits from open-air fresh markets and supermarkets throughout Bangkok, Thailand. These epidemiological data on bacterial AMR can be used for risk assessments of AMR contamination in raw produce and also help establish a baseline of contamination to then quantify future trends of AMR contamination subsequent to monitoring and surveillance. Recently, Thailand's national strategic plan for 2017–2022 established an AMR surveillance system under the One Health approach that will standardize and harmonize AMR surveillance protocols in human, animal, food, and environmental sectors, including crop production (e.g., vegetables and fruits). The occurrence of *E. coli* and *S. enterica* that harbored $bla_{\text{CTX-M-55}}$ with MDR indicates the possible foodborne transmission of AMR and MDR bacteria from fresh produce to humans, especially for produce commodities consume raw or minimally processed. It might be prudent to further investigate the dynamics of bacterial AMR dissemination and persistence in other environmental settings to prevent the spread of resistant *E. coli* and *S. enterica*, and in addition, practice safe hygiene, washing raw vegetables before consumption, and avoiding excessive use of antimicrobials in human and animal health to help prevent the spread of AMR.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Benjawan Saechue: Writing – original draft, Validation, Methodology, Formal analysis. **Edward R. Atwill:** Writing – review & editing, Supervision, Software. **Saharuetai Jeamsripong:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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