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Prevalence and Analysis of Antibiotic Resistant Genes in *Escherichia coli* and *Salmonella* Isolates from Green Leaf Lettuce

Chhay Chanseyha, Muhammad Bilal Sadiq, Tay Zar Aye Cho and Anil Kumar Anal*
Food Engineering and Bioprocess Technology, Asian Institute of Technology, Klong Luang, Pathumthani 12120, Thailand.
* Author for correspondence; e-mail: anilkumar@ait.asia; anil.anal@gmail.com

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ABSTRACT

The objective of this study was to evaluate the prevalence and antibiogram of *Escherichia coli* and *Salmonella* isolated from green leaf lettuce (n=120), collected from open and supermarkets of Cambodia and Thailand. From 120 samples of lettuce leaves, 47 (39.17%) *E. coli* and 28 (23.33%) *Salmonella* were isolated and identified by biochemical and immunological tests. Twenty seven *E. coli* isolates (57.45%) and 13 *Salmonella* isolates, (46.43%) were found resistant to at least one of the antibacterial drugs. *Escherichia coli* were more resistant to amoxicillin and ampicillin (92.6%), followed by tetracycline (70.4%). Almost all the isolated *Salmonella* were found resistant to ampicillin and amoxicillin, followed by tetracycline (69.23%). The beta-lactam (bla_{TEM}) and tetracycline (*tetA* and *tet*B) resistance genes were analyzed by polymerase chain reaction in the tested bacterial isolates. This study concludes that fresh vegetables and fruits should be subjected to pretreatment prior to human consumption, to avoid the foodborne illnesses associated with multidrug resistant bacteria. Moreover, the use of antibiotics in agriculture farming should be strictly monitored in developing countries to avoid the emergence of multidrug resistant pathogens.

Keywords: *Escherichia coli*, *Salmonella*, Green leaf lettuce, Antibiogram, Resistant gene

1. INTRODUCTION

Foodborne illnesses caused by various microorganisms including bacteria are major concern to public health that lead to morbidity and mortality in both developed and developing countries [1]. Foodborne diseases not only affect people's well-being, but also cause hospitalization and economical loss. Approximately 22.8 million cases of diarrheal illness were caused by Salmonellosis outbreak annually, with death of 37,600 in South East Asia [2]. Among foodborne pathogens, *E. coli* and *Salmonella* are the most common pathogens found in food and responsible for various diseases [3]. In fact, various research reports indicated the prevalence of *E. coli* in fresh lettuce leaves, fresh cut vegetables and ready to eat salads [4]. Food-borne pathogens are major cause of infectious diseases

outbreaks in developing and developed parts of the world [5]. The incidences of foodborne illnesses associated with consumption of fresh fruits and vegetables have rapidly increases during the past few decades [6]. The consumption of fresh produce causes 20 million illnesses every year in United States [7].

Generally, food contributes as an important part for transfer of antibiotic resistance in terms of antibiotic residues or resistant genes from food microflora to pathogenic bacteria [8]. In order to improve and implement food safety system, monitoring the prevalence of pathogenic bacteria along with antibiotic resistant foodborne pathogens in food chain is one of the major tasks. Fresh produces are normally colonized by a wide variety of spoilage and pathogenic microorganisms [4]. Gram-negative bacteria, especially the members of the *Pseudomonadaceae* and *Enterobacteriaceae* were normally found on contaminated lettuce leaves [9]. Lettuce (*Lactuca sativa*), mostly consumed in its raw form as salad without any processing, is an annual leafy vegetable and belongs to the family *Asteraceae*. However, this kind of leafy vegetable was considered as the reservoir and vehicle for transmitting bacterial, parasitic and viral illness to human [4]. As the association between lettuce and foodborne disease outbreaks has been increasing, it has led to concerns about contamination of vegetables with fecal pathogenic bacteria in farms and the markets. These contaminations occur during pre-harvest, harvest and post-harvest stages due to soil, fresh manure fertilizer, irrigation water, wild and domestic animals, human handling and during display in market [10]. *E. coli* and *Salmonella* generally cause a self-limiting illness; however, antibiotics are required in severe conditions that may occur in some patients and animals [11].

In recent years, bacterial resistance to antibiotics has increased significantly [12]. The antimicrobial drug resistance among foodborne pathogens has increased due to extreme use of antibiotics in livestock, aquaculture and agriculture farming for therapeutic and prophylactic purposes [13]. Resistant strains of *E. coli* and *Salmonella* are generally transferred from animal feed, humans and fresh produces through the food chain. In addition, plasmid-encoded resistant genes are generally transferred from one pathogen to the other, which potentially result in increased antibiotic resistance in natural ecosystem [14].

The objective of this study was to reveal the prevalence of *E. coli* and *Salmonella* in green leaf lettuce and to evaluate the resistance and susceptibility profile to commercially available antibiotics and identification of antimicrobial resistant genes. No detailed study has been conducted on antibiotic resistance in fresh produce vegetables in Cambodia and Thailand.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples of green leaf lettuce (n=120) were collected from open and supermarkets of Phnom Penh city, Cambodia and Pathumthani Province, Thailand. The samples were placed in sampling box containing ice pads and transported to Bioprocess Technology laboratory at the Asian Institute of Technology (AIT), Pathumthani, Thailand. All the samples were provided identification code with respect to the areas of sample collection as; open market Thailand (OMT), super market Thailand (SMT), open market Cambodia (OMC) and super market Cambodia (SMC).

2.2 Sample Preparation and Enrichment

Lettuce leaves were first cut aseptically

with sterile scissors and forceps and each sample (25 g) was transferred to 225 mL of buffered peptone water (BPW, HiMedia, India), followed by homogenization with stomacher device (BAGMIXER400W, Interscience, France). The mixtures were then incubated at 37°C for 24 h. Following the incubation, pre-inoculated BPW was used for isolation of *E. coli* and *Salmonella*.

2.3 Isolation and Identification of *E. coli* and *Salmonella*

Pre-inoculated BPW (1 mL) was transferred to 9 mL of selective enrichment broth media (EC, HiMedia, India) and incubated further at 37°C for 24 h. A loopful of the EC broth was streaked on Eosin Methylene Blue agar (EMB, HiMedia, India) and incubated at 37°C for 24 h. Presumptive colonies of *E. coli*, which appeared dark purple color with green metallic sheen, were further streaked on Nutrient Agar (NA, HiMedia, India). After incubation at 37°C for 24 h, isolated colonies were subjected to biochemical tests (triple sugar iron agar test, indole test, methyl red test, Voges Proskauer test and citrate test). Further confirmation for *E. coli* was carried out by serological tests (polyvalent antisera "O" and "H") [15].

Similarly, pre-inoculated BPW (1 mL) was transferred to 9 mL of selective enrichment media Rappaport vassiliadis soyabean broth (RVS, HiMedia, India) and Tetrathionate broth (TTB, HiMedia, India) for identification of *Salmonella*, followed by incubation at 37°C for 24 h. After incubation of 24 h, a loopful of each, RVS and TTB were streaked on xylose lysine deoxycholate agar (XLD, HiMedia, India) and bismuth sulfite agar (BSA, HiMedia, India) and further incubated at 37°C for 24 h. Presumptive *Salmonella* colonies which appeared pink/red with black centers on XLD agar and brown, gray or black colonies with metallic sheen on BSA were streaked on Nutrient Agar (NA, HiMedia, India). After further incubation at 37°C for 24 h, isolated colonies were subjected to biochemical tests. *Salmonella* were further confirmed by serological tests (polyvalent antisera "O" and "H") [8].

2.4 Antibiotic Susceptibility Testing of *E. coli* and *Salmonella*

The antibiogram profile of *E. coli* and *Salmonella* were determined by using disk diffusion method following the guidelines of Clinical and Laboratory Standard Institute [16]. The antibiotics that are commonly used in humans and agriculture practices for treatment and prophylactic purposes were selected: ampicillin (10 µg), amoxicillin (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), trimethoprim (5 µg), tetracycline (30 µg), and ciprofloxacin (5 µg) (HiMedia, India). *E. coli* and *Salmonella* isolates were sub-cultured in nutrient broth at 37°C for 18 h and adjusted to 0.5 McFarland standard (10^8 CFU/mL). The bacterial suspensions were then inoculated on Mueller-Hinton agar (HiMedia, India) followed by placing the antibiotic disks with the help of sterile forceps. The agar plates were further incubated at 37°C for 24 h. The diameter of inhibition zone around each antibiotic disk was measured in millimeter and based on inhibition zone; bacterial isolates were classified as susceptible, intermediate or resistant according to the CLSI criteria for *Enterobacteriaceae*.

2.5 Detection of Antibiotic Resistance Genes in *E. coli* and *Salmonella* Isolates

The set of primers (Table 1) for beta-lactams (bla_{TEM}, bla_{SHV}, bla_{CMY}), tetracycline [*tet(A)*, *tet(B)*], gentamicin (*aac(3)-IV*), streptomycin (*aadA1*), ciprofloxacin (*gyrA*), trimethoprim (*dhfrI*), and chloramphenicol

(*catA1*, *cmlA*) were acquired from Sigma Aldrich, Singapore. The genomic DNA was extracted from overnight grown cultures of *E. coli* and *Salmonella* by using genomic DNA purification kit (Insta-max gene matrix Bio-Rad, USA) according to instructions provided by the manufacturer. The PCR reactions were performed in a total volume of 25 µL, including 1 µL of each, reverse and forward primer (10 µM), PCR grade water 1.5 µL, Taq PCR master mix (Bio-Rad, USA) 12.5 µL and 9 µL of DNA (100-200 ng/µL). Amplification reactions were accomplished by using a DNA thermo-cycler (Bio-Rad) as follows: initial denaturation for 30 seconds at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing for 60 seconds (59°C, 61°C and 65°C for bla_{TEM}, *tet(A)* and *tet(B)*, respectively) [21]. After final extension for 5 min at 68°C, amplified samples were subjected to gel electrophoresis by using QIAxcel advance system (QIAGEN, USA).

Table 1. Genes, primer sequences and conditions used for PCR analysis of antibiotic resistant genes in *E. coli* and *Salmonella*.

Antibiotic	Resistance gene	DNA Sequence 5′ to 3′	Size (bp)	Annealing temperature (°C)	Reference
Beta-lactams (Ampicillin and Amoxicillin)	bla_{TEM}	(F) TTGCCGGGAAGCTAGAGTAA (R) GAGGACCGAAGGAGCTAACC	202	59°C	[1]
	bla_{CMY}	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	325	65.2°C	[17]
	bla_{SHV}	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	462	52°C	[17]
Gentamicin	*aac(3)-IV*	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	55°C	[1]
Streptomycin	*aadA1*	(F) TATCCAGCTAAGCGCGAACT (R) ATTTGCCGACTACCTTGGTC	447	58°C	[17]
Tetracycline	*tet (A)*	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	662	61°C	[18]
	tet (B)	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	730	65°C	[18]
Ciprofloxacin	*gyrA*	(F) AAATCTGCCCGTGTCGTTGGT (R) GCCATACCTACGGCGATACC	343	53°C	[19]
Trimethoprim	*dhfrI*	(F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC	367	50°C	[20]
Chloramphenicol	*catA1*	(F) AGTTGCTCAATGTACCTATAACC (R) TTGTAATTCATTAAGCATTCTGCC	547	55°C	[1]
	cmlA	(F) CCGCCACGGTGTTGTTGTTATC (R) CACCTTGCCTGCCCATCATTAG	698	55°C	[1]

F - forward primers; R - reverse primers

bp - base pairs

2.6 Statistical Analysis

The results were analyzed by chi-square (v2) and two-sided Fisher's exact tests by using SPSS statistical software package (SPSS, version 16.0). Results with $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Prevalence of *E. coli* and *Salmonella* Isolates

In this study, green lettuce leaves (n =120) were collected from open and supermarkets of Cambodia and Thailand. Out of 120 samples, 47 (39.17%) were found positive for *E. coli* and 28 (23.33%) samples were positive for *Salmonella*. In open markets of Thailand and Cambodia, prevalence of *E. coli* and *Salmonella* was relatively higher compared ($p < 0.05$) to supermarkets as shown in table 2.

Table 2. Prevalence of *E. coli* and *Salmonella* in open and supermarkets of Thailand and Cambodia.

Sample	Isolate	OMT (n=30)	Prevalence (%)	SMT (n=30)	Prevalence (%)	p-Value
Green Leaf Lettuce	*E. coli*	11	36.67	10	33.33	0.702
	Salmonella	8	26.67	4	13.33	0.454
	Isolate	OMC (n=30)	Prevalence (%)	SMC (n=30)	Prevalence (%)	p-Value
	E. coli	14	46.67	12	40	1.00
	Salmonella	10	33.33	6	20	1.00

Significant at $p < 0.05$ based on comparison between open market samples and super market. **OMT** - Open market Thailand, **SMT** - Supermarket Thailand, **OMC** - Open market Cambodia, **SMC** - Supermarket Cambodia.

In Thailand and Cambodia, open markets are quite popular among the consumers as it provides low-cost fresh vegetables, raw poultry products, meat, fish and ready-to-eat foods. But the risk of contamination by potential foodborne hazards is high in open markets [22]. Outbreaks of foodborne diseases associated with the consumption of raw food products have been increasing over the past few decades that resulted in a potential risk to public health [23]. The pathogens present in irrigation water can contaminate fresh produce and subsequently can cause disease outbreak in humans. Developing countries in which irrigation with untreated or insufficiently treated wastewater is common, the incidences of fruits and vegetables contamination are higher compared to developed countries [24]. The prevalence of *Salmonella* (28 isolates, 23.33%) was found less as compared ($p < 0.05$) to *E. coli* (47 isolates, 39.17%) in fresh lettuce leaves from open and supermarkets of Thailand and Cambodia. The results corroborate with the previous research reports, indicating the less prevalence of *Salmonella* from green leafy vegetables (3%) in Spain and salads (1.3%) in Brazil [4, 25]. The absence or low prevalence of *Salmonella* isolates from fresh produces in developed countries might be due to good agricultural practices and safety control programs. Vegetables contamination can occur at any time during their production, harvesting,

processing and preparation for consumption. Furthermore, the prevalence of *E. coli* and *Salmonella* was less in supermarket samples compared to samples from open markets. Supermarkets have better management and safety measures of vegetables including proper cleaning, packaging etc.

3.2 Antibiotic Susceptibility Testing of *E. coli* and *Salmonella* Isolates

Antimicrobial resistance has been known as the emerging issue in humans, animals and agriculture due to extensive use of antibiotics and the resistant strains of bacteria are likely to enter from environment to food chain, animals and humans [26]. The susceptibility and resistance patterns of *E. coli* and *Salmonella* isolates were determined against various commercially available antibiotics and results are shown in Tables 3 and 4.

Table 3. Antibiotic resistant profile of *E. coli* isolates from lettuce samples.

N°	Bacteria Code	Resistance	Susceptibility
1	OMT18	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP
2	OMT21	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP
3	OMT23	AMP, AMC, STR, TET, TMP	GEN, CIP
4	OMT26	AMP, AMC, STR, TET	GEN, TMP
5	OMT28	AMP, AMC, STR, TET, TMP	GEN, CIP
6	OMC4	AMP, AMC, TET	GEN, STR, CIP, TMR, CMP
7	OMC6	AMP, AMC	GEN, STR, TET, CIP, TMP, CMP
8	OMC8	AMP, AMC	GEN, STR, TET
9	OMC16	AMP, AMC, TMP, CMP	GEN, STR, TET, CIP
10	OMC21	AMP, AMC, CMP	GEN, TMP
11	OMC22	AMP, AMC, STR, TET	GEN, CIP, TMP
12	OMC25	AMP, AMC, TMP	GEN, CMP
13	OMC26	AMP, AMC, STR, TET	GEN, CIP, TMP
14	OMC27	AMP, AMC, TET, TMP	GEN, STR, CIP
15	OMC28	AMP, AMC, TMP	GEN, TET, CIP, CMP
16	SMT2	TET	AMP, AMC, GEN, STR, CIP TMP, CMP
17	SMT9	AMP, AMC	GEN, STR, CIP, TMP, CMP
18	SMT16	TET	AMP, AMC, GEN, STR, CIP TMP, CMP
19	SMT29	AMP, AMC	GEN, STR, CIP, TMP, CMP
20	SMC1	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP
21	SMC2	AMP, AMC, TET, TMP	GEN, STR, CIP
22	SMC6	AMP, AMC, TET	GEN, TMP
23	SMC11	AMP, AMC, TET, TMP	GEN
24	SMC16	AMP, AMC, TET	GEN, TMP
25	SMC20	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP
26	SMC24	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP
27	SMC28	AMP, AMC, TET	GEN, STR, CIP, TMP, CMP

OMT - Open market in Thailand, OMC - Open market in Cambodia, SMT - Super market in Thailand, SMC - Super market in Cambodia. Ampicillin - AMP, amoxicillin - AMC, chloramphenicol - CMP, streptomycin - STR, gentamicin - GEN, trimethoprim - TMP, tetracycline - TET, and ciprofloxacin - CIP

Table 4. Antibiotic resistant profile of *Salmonella* isolates of lettuce samples.

N°	Bacteria Code	Resistance	Susceptibility
1	OMT1	AMP, AMC, STR, TET, TMP	GEN, CIP
2	OMT8	AMP, AMC, STR, TET, TMP	GEN, CIP
3	OMC3	AMP, AMC, TET, TMP	GEN, STR, CIP
4	OMC6	AMP, AMC, TET	GEN, STR, CIP, CMP
5	OMC15	AMP, AMC, STR, TET	GEN, CIP, TMP
6	OMC16	AMP, AMC, TET, TMP	GEN, STR, CIP
7	OMC17	AMP, AMC, TET	GEN, TMP
8	SMC6	AMP, AMC	GEN, STR, TET, CIP, CMP
9	SMC7	AMP, AMC	GEN, STR, TET, CIP, CMP
10	SMC8	AMP, AMC, TET, TMP	GEN, CMP
11	SMC21	AMP, AMC	GEN, TET, TMP, CMP
12	SMC26	AMP, AMC	GEN, TET, TMP, CMP
13	SMC30	AMP, AMC, TET, TMP	GEN, STR, CMP

OMT - Open market in Thailand, OMC - Open market in Cambodia, SMC - Super market in Cambodia. Ampicillin - AMP, amoxicillin - AMC, chloramphenicol - CMP, streptomycin - STR, gentamicin - GEN, trimethoprim - TMP, tetracycline - TET, and ciprofloxacin - CIP

Among *E. coli* isolates (n = 47) from both countries, only 27 (57.45%) isolates were resistant to antibiotics, with 5 isolates from open market in Thailand, 4 isolates from supermarket in Thailand, 10 isolates from open market in Cambodia and 8 isolates from supermarket in Cambodia. In addition, out of 27 (57.45%) antibiotic resistant *E. coli* isolates, 25 (92.6%) were resistant to amoxicillin and ampicillin, 19 (70.4%) were resistant to tetracycline, 8 (29.63%) were resistant to trimethoprim, 5 (18.52%) were resistant to streptomycin and only 2 (7.41%) were resistant to chloramphenicol (Table 5).

Table 5. *E. coli* and *Salmonella* isolates resistant to antibiotics.

Antibiotic	*E. coli* (n = 27)	*Salmonella* (n = 13)
Ampicillin	25 (92.6%)	13 (100%)
Amoxicillin	25 (92.6%)	13 (100%)
Tetracycline	19 (70.4%)	9 (69.23%)
Trimethoprim	8 (29.63%)	6 (46.15%)
Streptomycin	5 (18.52%)	3 (23.08%)
Chloramphenicol	2 (7.41%)	NR
Gentamicin	NR	NR
Ciprofloxacin	NR	NR
Resistance to 1 class of antibiotic	6 (22.22%)	4 (30.77%)
Resistance to 2 classes of antibiotics	12 (44.44%)	2 (15.38%)
Resistance to ≥ 3 classes of antibiotics	9 (33.33%)	7 (53.85%)

NR - Non-resistant

In case of *Salmonella* isolates (n = 28) from both countries, only 13 (46.43%) isolates were resistant to antibiotics, with 2 isolates from open market in Thailand, 5 isolates from open market in Cambodia and 6 isolates from supermarket in Cambodia. In addition, out of 13 (46.43%) *Salmonella* isolates, 13 (100%) were resistant to amoxicillin and ampicillin, 9 (69.23%) were resistant to tetracycline, 6 (46.15%) were resistant to trimethoprim and only 3 (23.08%) were resistant to streptomycin (Table 5). Antibiogram patterns showed that *E. coli* (27 isolates) and *Salmonella* (13 isolates) were resistant to at least one of the tested antibiotics while some of these isolates were found multidrug resistant.

In this study, *E. coli* and *Salmonella* isolates from fresh leaf lettuce samples were found resistant to beta-lactams, tetracycline, trimethoprim and streptomycin antibiotic. These results corroborate with the previous reports indicating that *E. coli* and *Salmonella* isolates from vegetables were mostly resistant to tetracycline, ampicillin and streptomycin [27]. All *E. coli* and *Salmonella* isolates in this study were found susceptible to ciprofloxacin and gentamicin. Whereas, relatively less resistance of *E. coli* (7.41%) was found against chloramphenicol, but all *Salmonella* isolates were susceptible to chloramphenicol. This could be due to limited and banned use of these antibiotics in animals, food and agriculture products in both countries.

3.3 Detection of Antibiotic Resistance Genes in *E. coli* and *Salmonella* Isolates

All antibiotic resistant *E. coli* and *Salmonella* isolates were analyzed for antibiotic resistant genes using PCR. The antibiogram results were in accordance with the results of detection of resistant genes as shown in Table 6.

Table 6. Identification of antibiotic resistance genes in *E. coli* and *Salmonella* isolates.

		Number (%) of positive isolates[a]							
		Bla_{TEM}		Bla_{CMY}		*TetA*		*TetB*	
Country	Market	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*
Thailand	Open market	5/5	2/2	0/5	1/2	4/5	1/2	0/5	2/2
	Supermarket	1/2	ND	0/2	ND	0/2	ND	1/2	ND
	Total	6/7 (85.71)	2/2 (100)	0/7 (0)	1/2 (50)	4/7 (57.14)	1/2 (50)	1/7 (14.28)	2/2 (100)
Combodia	Open market	10/10	5/5	0/10	0/5	4/4	2/5	1/4	3/5
	Supermarket	4/8	3/6	0/8	0/6	4/8	0/2	0/8	1/2
	Total	14/18 (77.78)	8/11 (72.72)	0/18 (0)	0/11 (0)	8/12 (66.67)	2/7 (28.57)	1/12 (8.33)	4/7 (57.14)
Total		20/25 (80)	10/13 (76.92)	0/25 (0)	1/13 (7.69)	12/19 (63.15)	3/9 (33.33)	2/19 (10.52)	6/9 (66.67)

[a]Only antibiotic resistant isolates were tested for identification of resistant genes
ND - Non-detected

It was found that bla_{TEM} gene of beta-lactam (Figure 1a, 1b and 1c) and *tetA* and *tetB* of tetracycline resistant genes (Figure 2a and 2b) were frequently found in isolates of *E. coli* and *Salmonella*. However, bla_{SHV}, *aadA1*, *dhfrI*, *catA1*, and *cmlA* genes were not found in any of the *E. coli* and *Salmonella* isolates.

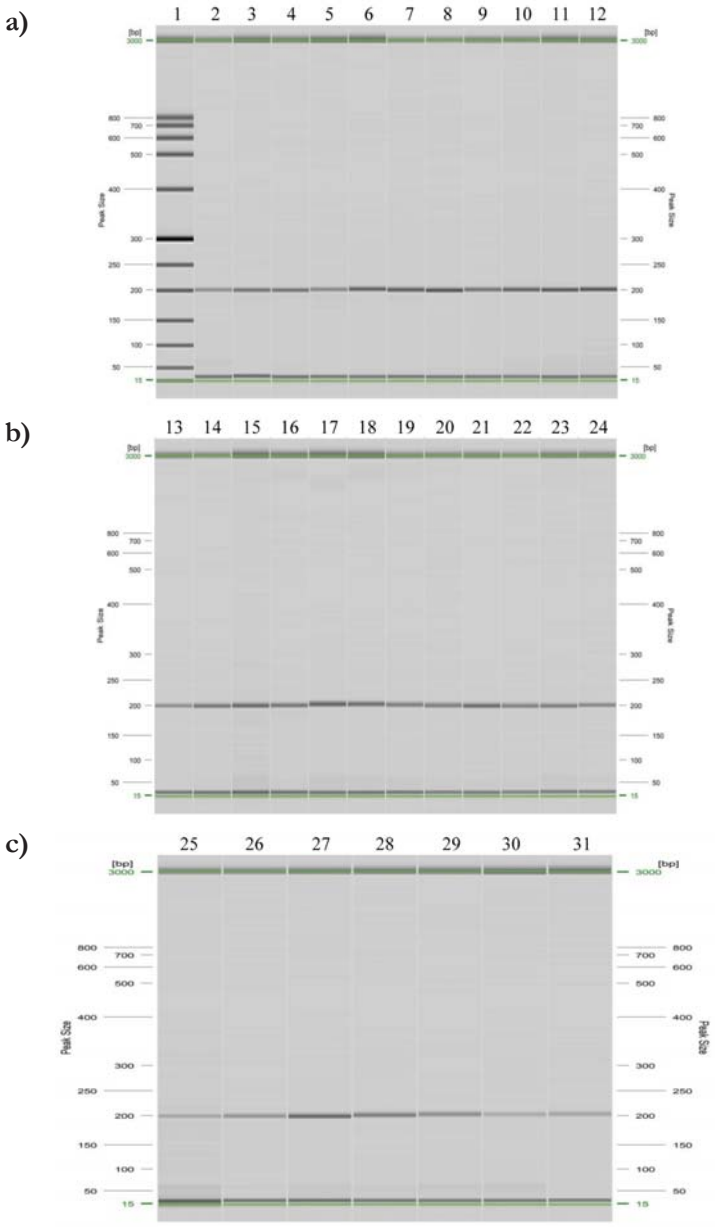

Figure 1. (a, b and c) PCR detection of bla_{TEM} gene in *E. coli* and *Salmonella* isolates
From lane 1 to 21 are bla_{TEM} gene coding for beta-lactam resistance *E. coli*
From lane 22 to 31 are bla_{TEM} gene coding for beta-lactam resistance *Salmonella*
Lane 1 - DNA Marker; lane 2 - 0MT18; lane 3 - OMT21; lane 4 - OMT23;
Lane 5 - 0MT26; lane 6 - OMT28; lane 7 - OMC4; lane 8 - OMC6; lane 9 - OMC8;
Lane 10 - OMC16; lane 11 - OMC21; lane 12 - OMC22; lane 13 - OMC25;
Lane 14 - OMC26; lane 15 - OMC27; lane 16 - OMC28 Lane 17 - SMT9;
Lane 18 - SMC1; lane 19 - SMC2; lane 20 - SMC6; lane 21 - SMC11;
Lane 22 - OMT1; lane 23 - OMT8; lane 24 - OMC3, lane 25 - OMC6;
Lane 26 - OMC15; lane 27 - OMC16; lane 28 - OMC17; lane 29 - SMC6;
Lane 30 - SMC7; lane 31 - SMC8

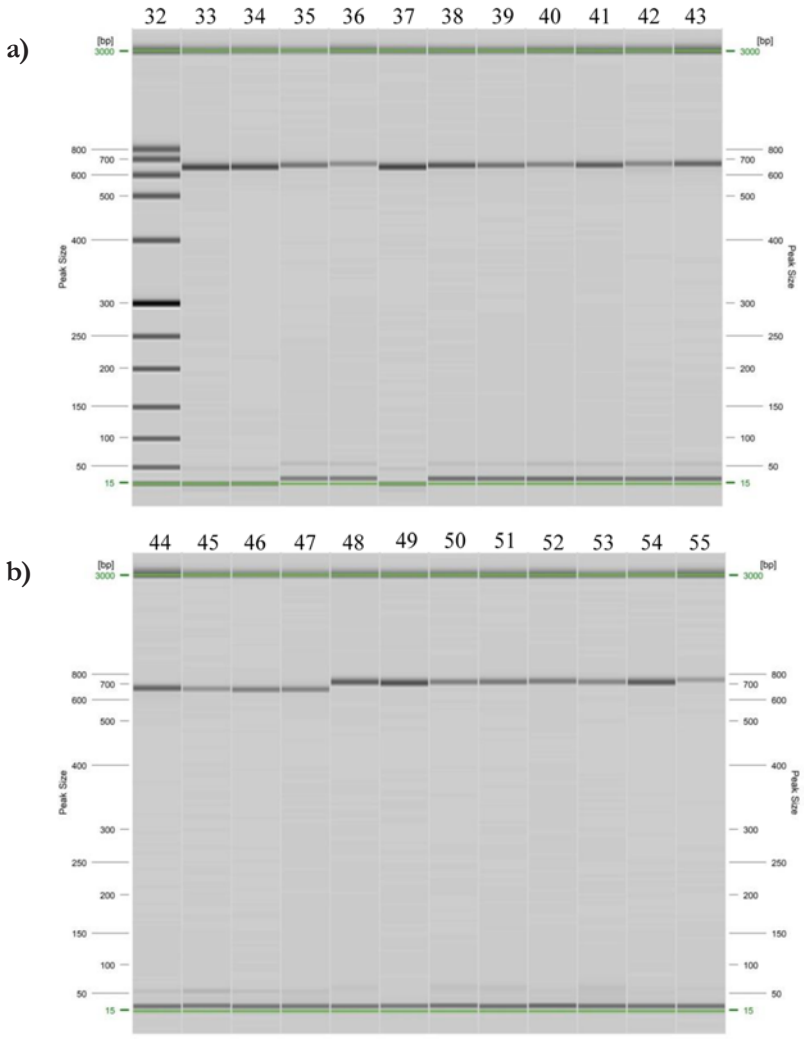

Figure 2. (a and b) PCR detection of *tetA* and *tetB* genes in *E. coli* and *Salmonella* isolates
From lane 33 to 44 are *tetA* gene coding for tetracycline resistance *E. coli*
From lane 45 to 47 are *tetA* gene coding for tetracycline resistance *Salmonella*
From lane 48 and 49 are *tetB* gene coding for tetracycline resistance *E. coli*
From lane 50 to 55 are *tetB* gene coding for tetracycline resistance *Salmonella*
Lane 32 - DNA Marker; lane 33 - OMT21; lane 34 - OMT23; lane 35 - OMT26;
Lane 36 - OMT28; lane 37 - OMC4; lane 38 - OMC22; lane 39 - OMC26;
Lane 40 - OMC27; lane 41 - SMC1; lane 42 - SMC2; lane 43 - SMC6;
Lane 44 - SMC11; lane 45 - OMT1; lane 46 - OMC3; lane 47 - OMC6
Lane 48 - OMC27; lane 49 - SMT2; lane 50 - OMT1; lane 51 - OMT8
Lane 52 - OMC6; lane 53 - OMC15; lane 54 - OMC16; lane 55 - SMC8

Overall, 20 (80%) out of 25 beta-lactam resistant *E. coli* showed the presence of bla_{TEM} gene. In case of beta-lactam resistant *Salmonella*, 10 (76.92%) out of 13 isolates were detected with bla_{TEM} gene and only one (7.69%) beta-lactam resistant *Salmonella* from open markets in Thailand was found positive for bla_{CMY} gene (Table 6).

Among tetracycline resistant *E. coli* isolates, 12 (63.15%) out of 19 isolates were positive for the detection of *tetA* gene, while only 2 (10.52%) isolates were positive for *tetB*. In case of tetracycline resistant isolates of *Salmonella*, only 6 (66.67%) out of 9 isolates were positive for *tetB* gene and 3 (33.33%) isolates were positive for *tetA* gene (Table 6).

Among resistant isolates, bla_{TEM} gene, responsible for resistance to β-lactam antibiotics was most abundant in *E. coli* (80%) and *Salmonella* (76.92%). Similar results were reported by Sheikh et al. [28] and Kim and Woo [29], indicating that *E. coli* isolates from vegetables and meat sources showed the positive detection of bla_{TEM} gene. *Bla* gene is responsible for plasmid mediated resistance by producing β-lactamases that hydrolyze β-lactam ring [30], reduced permeability of the beta-lactam antibiotics or increased efflux [31].

In addition, tetracycline resistant bacterial isolates were tested for the prevalence of *tet(A)* and *tet(B)*. Among *E. coli* isolates *tet(A)* gene was most frequent compared to *tet(B)*, whereas, *tet(B)* gene was more common among *Salmonella* isolates. The *tet(A)* and *tet(B)* genes are mostly responsible for encoding resistant to tetracycline among *E. coli* and *Salmonella* isolated from food sources [28-29]. Tetracycline resistant genes mediate the resistance mainly by up-regulation of efflux pumps, ribosomal protection proteins (dislodge tetracycline from binding ribosome), and inactivate enzyme [32-33].

The prevalence of antibiotic resistance genes was generally correlated with the phenotypic resistance but phenotype and genotype resistances could not be matched in various cases [34]. In this study, some bacterial isolates were resistant to trimethoprim, streptomycin and chloramphenicol, but corresponding tested resistant genes were absent that could be explained by the fact that phenotypic resistance patterns might be different from genotypic resistance or the selected sequence of primers may not match with the genes encoded for resistance in the bacterial isolates [27].

4. CONCLUSION

E. coli and *Salmonella* were found widely in fresh green leaves lettuce obtained from the open markets and even supermarkets of Cambodia and Thailand. Many bacterial isolates were resistant to commercially available antibiotics. The isolates were resistant to beta-lactam antibiotic, followed by tetracycline, trimethoprim and streptomycin. Most of the bacterial isolates carried beta-lactam (bla_{TEM}) genes and tetracycline (*tetA*), (*tetB*) genes that can be a potential threat for transmission of resistance from bacteria to human or even to other bacteria. Thus, food safety and control system implementation, and limitation in usage of antibiotics in animals, agriculture and human are required in urgent. Furthermore, practicing good hygiene from farm to fork and using natural antimicrobials are encouraged to cope with the current issue and to ensure the safety of fresh food.

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