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ORIGINAL ARTICLE

Screening of antibiotic resistance genes in pathogenic bacteria isolated from tiny freshwater shrimp (*Macrobrachium lanchesteri*) and "Kung Ten", the uncooked Thai food

Kanittada Thongkao, Yuttana Sudjaroen
Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand

ABSTRACT

Objective: This study aimed to isolate and identify of pathogenic bacteria in tiny freshwater shrimp (*Macrobrachium lanchesteri*) and in Kung Ten, which is an unusual Thai cuisine that eaten alive shrimp directly. Antimicrobial susceptibility test and identification of antibiotic resistance genes for isolated bacteria were conducted.

Materials and Methods: Eighty of fresh shrimp samples and forty of Kung Ten salads were collected from four fresh markets, which were located in Bangkok and Nonthaburi province (*N* = 120). The isolation, identification, and antimicrobial susceptibility test of pathogenic bacteria were done following the Clinical and Laboratory Standards Institute guidelines. Antibiotic-resistant bacteria were screened for β-lactamase relating genes, such as *Amp*C (*MOX* and *ACC* genes), $bla_{CTX\text{-}M}$, and *Int1* genes.

Results: The number of bacterial isolates in tiny freshwater shrimp and Kung Ten salad was 136 and 65, respectively. *Aeromonas caviae*, *A. hydrophilla*, *Proteus penneri*, *Proteus vulgaris*, and *Klebsiella pneumoniae* were commonly found. Ampicillin, amoxicillin/clavulanic, cefuroxime, tetracycline, and trimethoprim/sulfamethoxazole resistance were observed, and common antibiotic-resistant bacteria were *A. caviae*, *P. vulgaris*, *Enterobacter Aerogenes*, and *K. pneumoniae*. *A. caviae*, *P. penneri*, *K. Pneumoniae*, and *A. hydrophilla* were positive for *MOX* gene; $bla_{CTX\text{-}M}$, and *Int1* genes; *ACC* and *Int1* genes; and *ACC* gene, respectively.

Conclusion: Raw or uncooked shrimps in Kung Ten salad may a risk in foodborne diseases due to positive for pathogenic bacterial isolates. However, hygienic control on food preparation is difficult to apply because of the difficulty of changing in local Thai food behavior.

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Introduction

The consumption of raw or half-cooked meat and fishery products may cause human illness. Most of the causative agents of foodborne diseases are associated with bacterial contamination. Risk of symptoms is common in the gastrointestinal tract; however, there are rarely infected in other systems of human body. According to World Health Organization disease control, zoonosis is transmitted from animals to humans and commonly infected via contaminated food [1].

The bacterial contamination can remain in the food processing area as biofilm formation, which can be spread to food processing equipment and other food-contact surfaces [2]. Cholera, diarrhea, gastroenteritis, and septicemia are leading epidemic symptoms of foodborne illness [3]. Significant pathogenic bacteria including *Salmonella*, *Campylobacter*, *Shigella*, *Listeria*, and *Escherichia coli* O157:H7 are commonly tracked by the food net and responsible for most of the infection cases [4]. In the case of aquatic animals and fishery products, the examples of foodborne pathogenic bacteria included *Vibrio cholerae, Salmonella* spp.*, V. parahaemolyticus, Aeromonas* spp.*, L. monocytogenes, Staphylococcus aureus, Clostridium botulinum, C. perfringens, Edwardsiella tarda*, and *Shigella* spp., which had been reported in [5].

Correspondence Kanittada Thongkao ✉ kanittada@ssru.ac.th Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, Thailand.

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Shrimp is one of the fishery foods and has carry with pathogenic bacteria; however, the reports do not provide information on surveillance data or outbreak on the type of products especially when the product is prepared and consumed at private homes or specific cultural style. The increment of risk from pathogen-contaminated shrimp to consumers is unclear; however, it may due to the distribution of antibiotic resistance genes (ARGs) has been implicated in bacterial community, which are hosted shrimp and leading to the presence of ARGs induced increasing horizontal gene transfer [6–8]. Understanding ARGs may provide in benefit for consumers because of the negative consequences that may affect inevitably our health system to decrease the risk of acquiring diarrheal disease [9]. For example, ARGs are emerging in *Pseudomonas aeruginosa* and *Bacteroides fragilis* and possess multidrug resistance. The common occurrence of β-lactams resistance in Gram-negative bacteria is β-lactamase production [10]. Class A of β-lactamase enzymes is mainly membering in extended-spectrum β-lactamases (ESBLs), which are encoding by various genes such as bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{PER}, and bla_{PSE}, and some of bla_{OXA} genes. Actions of *AmpC* β-lactamase in ESBL antibiotic resistance are drug effluxion and reducing porin protein; and the dectection of this enzyme is commonly set up in clinical laboratory use [11]. Recently, the application of multiplex polymerase chain reaction (PCR) for *V. harveyi*, *V. parahaemolyticus*, and *V. Vulnificus* detections had evaluated in pacific white shrimp (*Litopenaeus vannamei*) and Thai shellfishes [7,8]. In addition, the dection of β-lactamase relating genes in *Klebsiella pneumoniae*, which were isolated from visceral organs of Tilapia fishes (*Oreochromis niloticus*) had been reported [7,8,12].

Tiny freshwater shrimp (*Macrobrachium lanchesteri*) is a small aquatic crustacean (15–56 mm), rostrum straight or weakly curving dorsal, barely reaching or extending slightly beyond antennal scale, usually 8 to 9 of dorsal and usually 3 to 4 of ventral teeth [13]. Their productivity is part of the food chain that may be an important economic in human food and aquaculture. The human consumption can be cooking by many Thai recipes; however, one of the most favors in Thai food, especially in North and North-East of Thailand is "Kung Ten" or freshwater shrimp spicy salad. Kung Ten salad is an unusual cuisine that most commonly known as "Dancing shrimp salad" due to the use of living tiny shrimp prepared with spices and vegetables and eating them directly when they are still alive. Thus, Kung Ten is a high risk for foodborne bacterial infection by uncooked recipes. However, rarely report about bacterial contamination in tiny freshwater shrimp and Kung Ten salad had reported. As this standing point, we are concerned about pathogenic bacterial contamination of shrimp in an uncooked shrimp salad. Thus, isolation, identification, and antimicrobial susceptibility of pathogenic bacteria were conducted and were further screened for β-lactamase relating genes, such as *AmpC* (*MOX* and *ACC* genes), $bla_{CTX\text{-}M}$, and *Int1* genes.

Materials and Methods

Ethical approval

Ethical approval of research protocol was considered and certified as an exemption for research ethics (COE. 1-007/2019) by Suan Sunandha Rajabhat Ethics Committee.

Sample collection and preparation

Tiny freshwater shrimps and dancing shrimp or "Kung Ten" salads were randomly purchased from four fresh markets in two districts of Bangkok, Thailand including Bang Phlat and Bangkok Noi; and two districts of Nonthaburi including Bang Yai and Muang. Twenty of fresh shrimp samples and Ten of Kung Ten salad were collected for each area (N = 120) during January–April, 2019. Identification of shrimps was characterized by reference data from the Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand; and confirmed by fishery specialist. One hundred grams (40–60 shrimps/each sample) was grinded and randomly to take a swab on transport medium on Stuart transport medium (Thermo Fisher Scientific, USA). The control of contamination in the sample collection process was using sterile technique. The transporting tubes were chilled with icepacks and sent back to laboratories for further bacterial cultivation within 6–8 h.

Bacterial isolation and identification

Transporting samples were subcultured for isolation and identification of pathogenic bacteria, which were carried out at the microbiological laboratory unit, Faculty of Science and Technology, Suan Sunandha Rajbhat University. Briefly, each sample was streaked and cultured with blood, MacConkey, and chocolate agars at 37°C, 24 h. Bacterial isolates were identified according to Bergey's Manual of Systematic Bacteriology, which included morphology of colony, Gram ,s staining and biochemical tests. The panel of biochemical tests, such as catalase, oxidase, coagulase, Triple Sugar Ion Agar, citrate, lysine indole motility, ornithine decarboxylase, methyl red- Vogeprokauer, Mannitol, growth 0% NaCl, growth 6.5% NaCl, and bile esculin tests, which were included in this study; and the interpretations of result for bacterial genus and species identification were according to the Clinical and Laboratory Standards Institute (CLSI) guideline [14].

Antimicrobial susceptibility testing

Each bacterial isolate was tested for antibiotic susceptibility, which was performed by the Kirby-Bauer method.

Briefly, the bacterial isolate was grown in Tryptic Soy Broth at 37°C for 3–4 h and concentration of culture suspension was adjusted at 0.5 McFarland turbidity and equal to 10^8 Colony Forming Unit (CFU)/ml. One hundred microliters of each culture medium was spread on the Müller-Hinton agar plate and then standing for 5 min for plate drying. Standard antibiotic disks were applied to spreaded plates (4 disks/plate) by antibiotic disk dispenser and incubated at 37°C for18–24 h. Thirteen of antibiotic disks (Difco, USA) were used for each test including amikacin, amoxicillin/clavulanic, ampicillin, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, imipenem, meropenem, tetracycline, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, and ertapenem. Results were interpreted by the diameter of the clear zone (mm), which surrounded with testing disk and the range of diameter was interpreted as susceptible (S), intermediate (I) and resistance (R) according to the CLSI recommendation [15].

DNA extraction and PCR technique

Bacterial isolates (1 × 10^9 CFU/ml or 1 loopful) were extracted by introducing a PrestoTM Mini gDNA bacteria kit (Geneaid Biotech, Taiwan) in 1.5 ml of Eppendorf microcentrifuge tube. Proteinase K (11 mg/ml) was added and incubated at 60°C for 10 min. Buffer lysate removal was done at 70°C for 10 min and the mixture was vortexed for 3 times for 1 min. The sample mixture was filled in and passed through the column, which was rinsed with cold 70% ethanol (Hanil Science Industrial, Korea) and the centrifuge at 14,000–16,000 gm. The DNA template was eluted by100 µl of pre-heated elution buffer.

PCR condition for $bla_{\text{CTX-M}}$ gene: 50-µl of the reaction mixture was contained 30 pmol of each primer, 200 µM concentrations of each deoxynucleoside triphosphate (Vivantis Technologies, Malaysia), 1.5 mM $MgCl_2$, (Vivantis Technologies, Malaysia), and 0.5 U of *Taq* DNA polymerase (Vivantis Technologies, Malaysia). CTX-MF (5′-ATG TGC AGY ACC AGT AAR GT-3′) and CTX-MR (5′-TGG GTR AAR TAR GTS ACC AGA-3′) primers (Theera trading, Thailand) were used to amplify a 693-bp of DNA template. PCR procedures were run as the following cycle, including initial denaturation of DNA at 94°C for 7 min, 35 cycles were run—94°C for 50 sec, 50°C for 40 sec, and 68°C for 1 min—with a 5-min 68°C extension after the 35 cycles [16].

PCR condition for class I integron (*Int1*) gene: *Int1*F (5′-GGG TCA AGG ATC TGG ATT CG-3′) and Int1R (5′-ACA TGG GTG TAA ATC ATC GTC-3′) was used as specific primers (Theera trading, Thailand). The reaction mixture was contained 25 pmol of each primer, 200 µM of each deoxynucleoside triphosphate, 1.5 mM of $MgCl_2$, and 0.5 U of *Taq* DNA polymerase was used to amplify 493-bp and 693-bp DNA templates. PCR was run as initial denaturation of DNA at 94°C for 5 min, 30 cycles were run—94°C for 30 sec, 62°C for 30 sec, and 72°C for 1 min—with an 8-min 72°C extension [17].

PCR condition for *MOX* and *ACC* genes: *AmpC* β-lactamases gene (*MOX* and *ACC* genotypes) primers, including MOXMF (5′-GCT GCT CAA GGA GCA CAG GAT-3′), MOXMAR (5′-CAC ATT GAC ATA GGT GTG GTG C-3′), ACCMF (5′-AAC AGC CTC AGC AGC CGG TTA-3′), and ACCMR (5′-TTC GCC GCA ATC ATC CCT AGC-3′), were used as specific primers, respectively [11]. The reaction mixture was contained 25 pmol of each primer, 200 µM of each deoxynucleoside triphosphate, 1.5 mM of $MgCl_2$, and 0.5 U of *Taq* DNA polymerase. The PCR program on an initial denaturation step at 94°C for 3 min, followed by 25 cycles of DNA denaturation at 94°C for the 30 sec, primer annealing at 64°C for 30 sec, and primer extension at 72°C for 1 min. After the last cycle, a final extension step at 72°C for 7 min.

Each DNA product of PCR mixture was separated by 1.5% agarose (Vivantis Technologies, Malaysia) gel electrophoresis (BIO-RAD, Thailand); stained with Novel Juice, DNA staining reagent (Bio-Helix (GibThai), Thailand). DNA electropherogram was took a photo under Biorad Universal Hood II Gel Doc System (BIO-RAD Laboratories-Segrate, Italy).

Data analysis

Descriptive and inferential statistics were analyzed the data by using MedCalc-Free statistical calculator (MedCalc, Ostend, Belgium). The values of continuous variables were presented as mean ± standard deviation (SD), whereas nominal variables were expressed as percentages and numbers. The difference of isolated bacterial number between freshwater shrimp and Kung-Ten salad was tested by an independent *t*-test. Statistical significant was judged at $p < 0.05$

Results and Discussion

The distribution of bacteria in tiny freshwater shrimp and Kung Ten salad was 136 and 65 isolates, respectively; and *Aeromonas caviae*, *A. hydrophilla*, *Proteus penneri*, *Proteus vulgaris,* and *K. pneumoniae* were commonly found from freshwater shrimp and Kung Ten salad in all study areas (Table 1). The three of most prevalent isolated bacteria from freshwater shrimps and Kung Ten salad were *A. caviae*, *P. penneri*, and *A. hydrophilla*, which were 70(38.7%), 43(23.6%), and 20(11.0%), respectively (Table 2). Ampicillin resistance has occurred in bacteria, which were isolated from tiny freshwater shrimp including *A. caviae*, *A. hydrophilla*, *P. penneri*, *P. vulgaris*, and *K. pneumoniae*; however, only *A. caviae*, *P. vulgaris*, and *Enterobacter aerogenes* isolated from Kung Ten salad were resisted to ampicillin.

Table 1. The distribution of bacterial identification from tiny freshwater shrimp and Kung Ten salad.

Sampling area	Bacterial identification	Number of isolates	
		Freshwater shrimp	Kung Ten salad
Bang Phlat, Bangkok	*A. caviae*	20	nd*
	Corynebacterium sp.	2	nd
	P. vulgaris	3	nd
	K. pneumoniae	3	nd
Bangkok Noi, Bangkok	*A. caviae*	20	-
	Corynebacterium sp.	1	1
	P. penneri	3	-
	K. pneumoniae	3	-
Bang Yai, Nonthaburi	*A. hydrophila*	20	-
	A. caviae	20	-
	Bacillus sp.	-	12
	Corynebacterium sp.	2	2
	K. pneumoniae	8	-
	E. aerogenes	1	2
	P. vulgaris	-	8
Muang, Nonthaburi	*A. caviae*	10	20
	P. penneri	20	20
Total of isolates		136	65

*nd = no detected or no growth of bacterial cultured

Table 2. The frequencies of bacterial species that isolated from tiny freshwater shrimp and Kung Ten salad.

Bacterial identification	Number of isolates		Total (%)
	Freshwater shrimp (N = 80)	Kung Ten salad (N = 40)	
A. caviae	50	20	70(38.7%)
P. penneri	23	20	43(23.6%)
A. hydrophila	20	-	20(11.0%)
K. pneumoniae	14	-	14(7.7%)
Bacillus sp.	-	12	12(6.6%)
P. vulgaris	3	8	11(6.1%)
Corynebacterium sp.	5	3	8(4.4%)
E. aerogenes	1	2	3(1.6%)
Statistical analysis			
Mean(±SD)	14.5(±16.81)	8.125(±8.39)	22.6(±22.64)
Independent *t*-test	95% CI = −11.96 to −0.78	t-statistic = −2.259	p = 0.0257*

* Statistical significance at $p < 0.05$

Amoxicillin/clavulanic was resisted by *E. aerogenes* from both isolated sources. Cefuroxime resistance has occurred in *P. vulgaris*, *P. penneri*, and *K. pneumoniae*, which were isolated from freshwater shrimp. Tetracycline and trimethoprim/sulfamethoxazole resistance were presented in *A. caviae*, and most of the antibiotic-resistant bacteria were *A. caviae*, *P. vulgaris*, *E. aerogenes*, and *K. pneumoniae*, respectively (Tables 3 and 4). All isolated

Table 3. Antimicrobial susceptibility pattern of bacteria isolates from tiny freshwater shrimp.

Antibiotic drugs	Bang Phlat, Bangkok			Muang, Nonthaburi			Bang Yai, Nonthaburi			Bangkok Noi, Bangkok			
	Aeromonascaviae	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Aeromonashydrophilla*	*Klebsiella pneumoniae*	*Enterobacter aerogenes*	*Aeromonas caviae*	*Proteus penneri*	*Proteus penneri*	*Aeromonas caviae*	*Aeromonascaviae*	*Proteus penneri*	*Klebsiella pneumoniae*
Amikacin (30 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Amoxicillin/clavulanic [30 mcg, (20/10 µg)]	-	S	S	-	S	R	-	S	S	-	-	S	S
Ampicillin (10 µg)	R	R	R	R	R	R	S	R	S	R	R	R	R
Ceftazidime (30 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Ceftriazone (30 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Cefuroxime (30 µg)	-	R	S	-	S	S	-	S	R	-	-	S	R
Chloramphenical (30 µg)	S	-	-	S	-	-	S	-	-	S	S	-	-
Ciprofloxacin (5 µg)	S	S	S	S	-	S	S	S	S	S	S	S	S
Gentamicin (10 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Imipenem (10 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Meropenam (10 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S
Tetracycline (30 µg)	S	-	-	S			S	-	-	S	R	-	-
Trimethoprim/sulfamethoxazole [25 µg (1.25/23.75 µg)]	S	S	S	S	S	S	S	S	S	S	R	S	S
Piperacillin/tazobactam (100 µg)	-	S	S	-	I	S	-	-	-	-	-	I	S
Ertapenem (10 µg)	-	S	S	-	S	S	-	S	S	-	-	S	S

bacteria were analyzed for detection of β-lactamase relating ARGs including *AmpC* (*MOX* and *ACC* genes), bla_{CTX-M}, and *Int1* genes. The electrophoresis separation of PCR product was positive for the detection of *AmpC* (*MOX* and *ACC* genes), bla_{CTX-M}, and *Int1* genes (Fig. 1). The detection of β-lactamase relating ARGs was 1) *A. caviae* was positive for *MOX* gene; 2) *P. penneri* was positive for bla_{CTX-M} and *Int1* genes; 3) *K. pneumoniae* was positive for *ACC* gene and *Int1* genes; and 4) *A. hydrophilla* was positive for *Int1* gene (Table 5).

Many members of the Enterobacteriaceae are antibiotic resistance, which may due to plasmid-mediated *AmpC* β-lactamases and are encoded on chromosomes [18–20]. *P. penneri* was resisted to cefuroxime and amoxicillin; however, it was commonly sensitive or intermediate susceptibility to tetracycline. There is also sensitive to aminopenicillins with β-lactamase inhibitors and some cephalosporins (cefazoline, cefuroxime, cefotiam, and cefdinir) [21,22].

Tetracycline and trimethoprim/sulfamethoxazole resistance in *A. caviae* have corresponded to the previous study; however, some strains of bacteria from the United States and Australia were also reported for more antibiotic resistance, including tetracycline, trimethoprim-sulfamethoxazole, cephalosporins, and aminoglycosides [23]. In this study, ampicillin, tetracycline, and trimethoprim/sulfamethoxazole resistance of *A. caviae* may be involved with the *MOX-6* gene (bla_{MOX-6}). Corresponding to the previous study, *Aeromonas hydrophila* and *A. caviae* were expressed ESBL characteristics and possess chromosomal *AmpC* β-lactamase genes [24]. *Proteus penneri* isolates are the most exhibited high resistance ampicillin, narrow-spectrum cephalosporins; and cefuroxime by the production of β-lactamase commonly known as cefuroximase. ESBL positive and multidrug-resistant (MDR) *P. penner*i was isolated from the pus, urine, and body fluid, and antibiotic resistance had been reported [25]. *CTX-M* β-lactamases are new *plasmid-mediated ESBLs*, which were first reported from Japan [26]. In this study, *CTX-M* type *beta-lactamase*-producing P. *penneri*. Class 1 Integrons (*Integrase*) has carried an evolution and spread of MDR *genes* in *P.* mirabilis [26]. Performing PCR for analyzing ARGs and Class 1 Integrons of *A. hydrophilla* and *P. penneri* is involved with the conjugation of horizontal

Table 4. Antimicrobial susceptibility pattern of bacteria isolates from Kung Ten.

Antibiotic drugs	Bangkok			Nonthaburi		
	Aeromonas caviae	*Proteus vulgaris*	*Enterobacter aerogenes*	*Proteus penneri*	*Aeromonascaviae*	*Aeromonascaviae*
Amikacin (30 µg)	-	S	S	S	-	-
Amoxicillin/clavulanic [30 mcg (20/10 µg)]	-	I	R	S	-	-
Ampicillin (10 µg)	S	R	R	S	R	S
Ceftazidime (30 µg)	-	S	S	S	-	-
Ceftriazone (30 µg)	-	S	S	S	-	-
Cefuroxime (30 µg)	-	S	S	S	-	-
Chloramphenical (30 µg)	S	-	-	-	S	S
Ciprofloxacin (5 µg)	S	S	S	S	S	S
Gentamicin (10 µg)	-	S	S	S	-	-
Imipenem (10 µg)	-	S	S	S	-	-
Meropenam (10 µg)	-	S	S	S	-	-
Tetracycline (30 µg)	R	S	-	-	S	S
Trimethoprim/sulfamethoxazole [25 µg (1.25/23.75 µg)]	S	-	S	S	S	S
Piperacillin/tazobactam (100 µg)	-	S	-	-	-	-
Ertapenem (10 µg)	-	S	S	S	-	-

S = sensitive or susceptible; I = intermediate; R = resistance (R)

gene transfer in carrying integron and ARGs between *A. hydrophilla* and *P. penneri*.

Moreover, the plasmid-mediated *AmpC* β-lactamases of co-transfer of antimicrobial resistance elements with integron are emerging and distributing of virulence factors among pathogenic bacteria of mobile genetic elements that have increasing public health problems in future [27,28].

Our finding corresponds to the previous study that reported ESBL and *AmpC* genes in Enterobacteriaceae bacteria isolated from raw seafood samples and the isolates mainly harboring resistance genes, including $bla_{\text{CTX-M}}$ (27.3%), bla_{CMY} (21.2%), or bla_{DHA} (21.2%), respectively. The increment of ESBL- and *AmpC*-producing Enterobacteriaceae prevalence in seafood trended to concern in public health management because of the potential transmission of these bacteria from food to humans [29,30].

The impact of antimicrobial resistance on Thai people is affected by health and economic being [31,32]. Various studies of antibiotic-resistant bacteria from patients, hospitals, community, animals, and foods had been reported for ESBL producing Enterobacteriaceae and carbapenem-resistant Enterobacteriaceae, which are common types of antibiotic resistance in Thailand [33,34]. Antibiotic-resistant bacteria in the environment in Thailand had been reported and mainly transmitted by wastewater from hospitals and markets [35]. In addition, aquacultures and farming were sources of antimicrobial use and can cause transmission of antimicrobial resistance [36,37].

Thus, the inappropriate control on antibiotic use can cause the spreading of antibiotic resistance in environments and turn back to infect human especially by food consumption. In this study, a high prevalence of Enterobacteria with ARGs was observed in tiny freshwater shrimps and living shrimp salad that not only suggests a health risk and public health concern but also implicates raw fishery food as vehicles of their dissemination into the households. The major limitations of our study were used a less amount of samples and unable to collect data from shrimp farming place. In addition, we cannot use the disk diffusion method to evaluate the minimum inhibitory concentration. Further studies are required for the investigation of other ARGs in isolated pathogenic bacteria or in other sources of risked food.

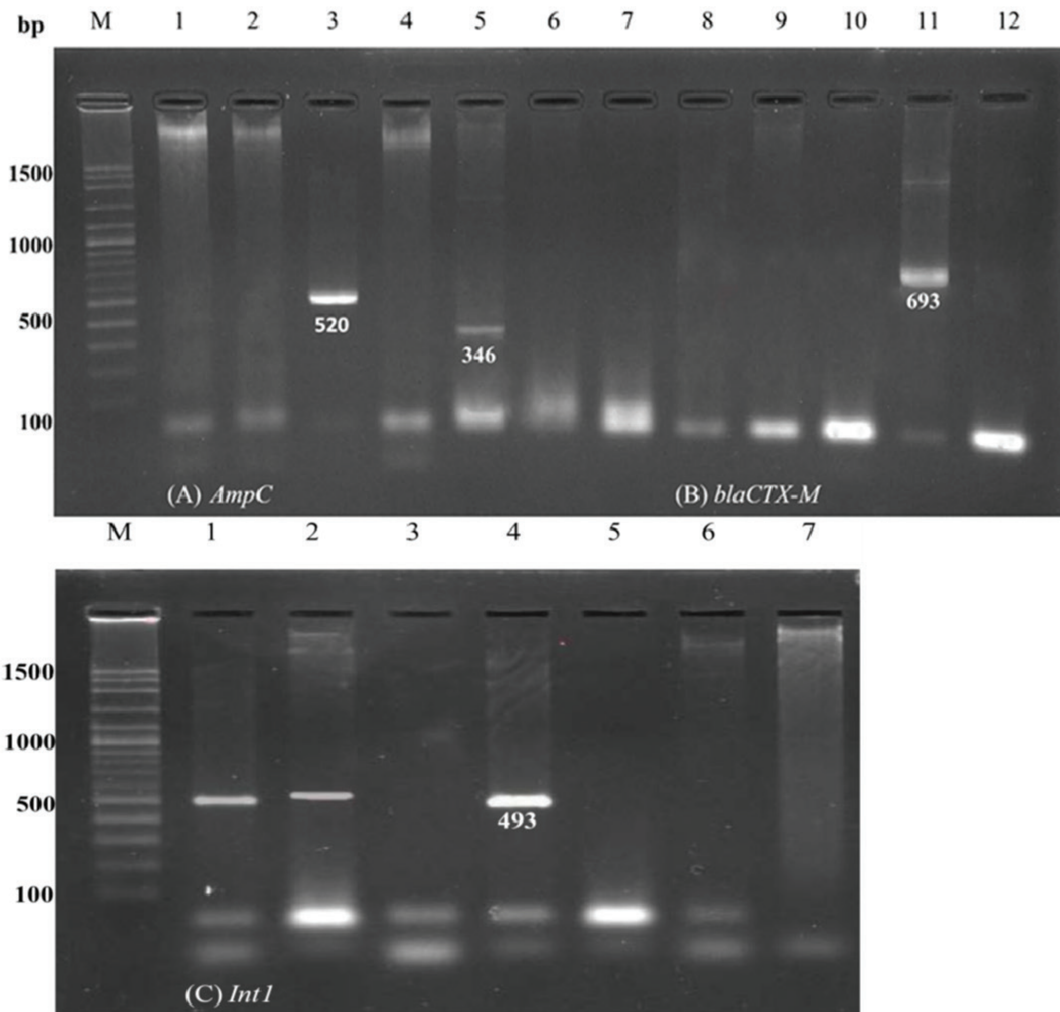

Figure 1. DNA electrophoresis separation of PCR product. PCR products amplification from different sources of bacterial culture identification, Lane M: DNA ladder (100 bp); (A) PCR product separation for *AmpC* genes (upper Lane 1–7): *Aeromonas caviae* (Bangkok Noi, Bangkok) was positive for *MOX* gene (upper Lane 3) and *Klebsiella pneumonia* (Bang Yai, Nonthaburi) was positive for *ACC* gene (upper Lane 5); (B) PCR product separation for *blaCTX-M* gene (upper Lane 8–12): *P. penneri* (Bang Yai, Nonthaburi) was positive for *blaCTX-M* gene (Lane 11); (C) PCR product separation for *Int1* gene (lower Lane 1-7): *A. hydrophila* (Muang, Nonthaburi), *K. pneumoniae* (Muang, Nonthaburi) and *P. penneri* (Bangkok Noi, Bangkok) were positive for *Int1* gene as shown in lower Lane 1, 2, and 4, respectively.

Table 5. The detection of *AmpC*, *bla*$_{CTX-M}$ and *Int1* genes in bacterial isolates.

Bacterial Species	*AmpC*		*bla*$_{CTX-M}$	*Int1*
	MOX	*ACC*		
A. caviae	+	-	-	-
P. penneri	-	-	+	+
A. hydrophila	-	-	-	+
K. pneumoniae	-	+	-	+
P. vulgaris	-	-	-	-
E. aerogenes	-	-	-	-

Conclusion

Raw or uncooked shrimps in Kung Ten salad may a risk in foodborne diseases due to positive for pathogenic bacterial isolates. Thus, the investigation of bacterial contamination with antibiotic resistance is needed to ensure the safety of food. However, hygienic control on food preparation is difficult to apply because of the difficulty of changing in local Thai food behavior.

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Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contribution

Kanittada Thongkao and Yuttana Sudjaroen were designed the research study and drafted the manuscript. Kanittada Thongkao was involved in laboratory works and data collection and also contributed in manuscript preparation. Yuttana Sudjaroen was interpreted in the summary focus of this study and critical checking of this manuscript.

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