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

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**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 AmpC (MOX and ACC genes), *bla* 

**Results:** The number of bacterial isolates in tiny freshwater shrimp and Kung Ten salad was 136 and 65, respectively. *Aeromonas caviae*, *A. hydrophila*, *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. hydrophila* were positive for MOX gene; *bla* 

**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.

**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, they 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 septi-cemia 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].

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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 benefit for consumers because of the negative consequences that may affect 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 blaTEM, blaSHV, blaCTXM, blaPER, and blaPSE and some of blaOXA genes. Actions of AmpC β-lactamase in ESBL antibiotic resistance are drug effluxion and reducing porin protein; and the detection 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. paraaeromlyticus, and V. Vulnificus detections had evaluated in pacific white shrimp (Litopenaeus vannamei) and Thai shellfishes [7,8]. In addition, the detection 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), blaCTXM, 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 Bangklok 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 Rajabhat 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-Vogesprokaueur, 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 10⁶ 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 spread plates (4 disks/plate) by antibiotic disk dispenser and incubated at 37°C for 18–24 h. Thirteen of antibiotic disks (Difco, USA) were used for each test including amikacin, amoxicillin/clavulanic, ampicillin, cefazidime, 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 × 10⁹ 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 by 100 μl of pre-heated elution buffer.

PCR condition for *blaCTX-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 MgCl₂ (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 amplified 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 *(IntI)* gene: IntI1 (5′-GGG TCA AGG ATC TGG ATT CG-3′) and Int1R (5′-ACA TGG GTG TAA ATC ATC GTC-3′) were 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 MgCl₂ 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′-CAG ATT GAC ATA GGT GTG GTG C-3′), ACCMF (5′-AAC AGC TCT AGC AGC CCG GTA-3′), and ACCMR (5′-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 MgCl₂, 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.
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 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  | Kung Ten salad |       |
|                          | (N = 80)           | (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)          | 95% CI = -11.96 to -0.78 | t-statistic = -2.259 | *p* = 0.0257* |
|--------------------------|--------------------|--------------------------|----------------------|---------------|

* Statistical significance at *p* < 0.05
bacteria were analyzed for detection of β-lactamase relating ARGs including AmpC (MOX and ACC genes), bla\textsubscript{CTX-M} and Int1 genes. The electrophoresis separation of PCR product was positive for the detection of AmpC (MOX and ACC genes), bla\textsubscript{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\textsubscript{CTX-M} and Int1 genes; 3) K. pneumoniae was positive for ACC gene and Int1 genes; and 4) A. hydrophilia 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 resited to cefuroxime and amoxicillin; however, it was commonly sensitive or intermediate susceptibility to tetracycline. There is also sensitive to amikacin, amoxicillin/clavulanic acid, and cefuroxime. ESBL positive and multidrug-resistant (MDR) P. penneri 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 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) have carried an evolution and spread of MDR genes in P. mirabilis [26]. Performing PCR for analyzing ARGs and Class 1 Integrons of A. hydrophilia and P. penneri is involved with the conjugation of horizontal

| Antibiotic drugs                    | Aeromonas caviae | Proteus vulgaris | Proteus mirabilis | Aeromonas hydrophilia | Proteus penneri | Klebsiella pneumoniae |
|-------------------------------------|-----------------|-----------------|------------------|----------------------|----------------|-----------------------|
| Amikacin (30 µg)                    | -               | S               | S                | S                    | S              | S                     |
| Amoxicillin/clavulanic acid (30 mcg, 20/10 µg) | -               | S               | S                | S                    | S              | S                     |
| Ampicillin (10 µg)                  | R               | R               | R                | R                    | S              | S                     |
| Cefazidime (30 µg)                  | -               | S               | S                | S                    | S              | S                     |
| Ceftiraxone (30 µg)                 | -               | S               | S                | S                    | S              | S                     |
| Cefuroxime (30 µg)                  | -               | S               | S                | S                    | S              | S                     |
| Chloramphenical (30 µg)             | S               | -               | -                | S                    | -              | -                     |
| Ciprofloxacin (5 µg)                | S               | S               | S                | S                    | S              | S                     |
| Gentamicin (10 µg)                  | -               | S               | S                | S                    | S              | S                     |
| Imipenem (10 µg)                    | -               | S               | S                | S                    | S              | S                     |
| Meropenam (10 µg)                   | -               | S               | S                | S                    | S              | S                     |
| Tetracycline (30 µg)                | S               | -               | -                | S                    | -              | -                     |
| Trimethoprim/sulfamethoxazole (25 µg, 1.25/23.75 µg) | S               | S               | S                | S                    | S              | S                     |
| Piperacillin/tazobactam (100 µg)    | -               | S               | S                | I                    | -              | -                     |
| Ertapenem (10 µg)                   | -               | S               | S                | S                    | S              | S                     |

http://bdvets.org/javar/
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*<sub>CTX-M</sub> (27.3%), *bla*<sub>CMY</sub> (21.2%), or *bla*<sub>DHA</sub> (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.

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

| Antibiotic drugs                          | Aeromonas caviae | Proteus vulgaris | Enterobacter aerogenes | Proteus penneri | Aeromonas caviae | Proteus vulgaris | Enterobacter aerogenes | Proteus penneri | Aeromonas caviae | Proteus vulgaris | Enterobacter aerogenes | Proteus penneri |
|-------------------------------------------|------------------|------------------|------------------------|-----------------|------------------|------------------|---------------------|-----------------|------------------|------------------|---------------------|------------------|
| Amikacin (30 μg)                          | -                | S                | S                      | S               | -                | S                | S                   | -               | -                | -                | -                   | -               |
| Amoxicillin/Clavulanic (30 mcg (20/10 μg)] | -                | I                | R                      | S               | -                | S                | S                   | -               | -                | -                | -                   | -               |
| Ampicillin (10 μg)                         | S                | R                | R                      | S               | S                | S                | -                   | -               | -                | -                | -                   | -               |
| Cefazidime (30 μg)                         | -                | S                | S                      | S               | -                | S                | S                   | -               | -                | -                | -                   | -               |
| Ceftriazone (30 μg)                        | -                | S                | S                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Cefuroxime (30 μg)                         | -                | -                | -                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Chloramphenical (30 μg)                    | S                | -                | -                      | -               | S                | S                | S                   | -               | -                | -                | -                   | -               |
| Ciprofloxacin (5 μg)                       | S                | S                | S                      | S               | S                | S                | S                   | -               | -                | -                | -                   | -               |
| Gentamicin (10 μg)                         | -                | S                | S                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Imipenem (10 μg)                           | -                | S                | S                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Meropenam (10 μg)                          | -                | S                | S                      | S               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Tetracycline (30 μg)                       | R                | S                | -                      | -               | S                | S                | S                   | -               | -                | -                | -                   | -               |
| Trimethoprim/Sulfamethoxazole [25 μg (1.25/23.75 μg)] | S                | -                | S                      | S               | S                | S                | S                   | -               | -                | -                | -                   | -               |
| Piperacillin/Tazobactam (100 μg)           | -                | S                | -                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |
| Ertapenem (10 μg)                          | -                | S                | S                      | -               | -                | -                | -                   | -               | -                | -                | -                   | -               |

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

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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Table 5. The detection of AmpC, blaCTX-M and Int1 genes in bacterial isolates.

| Bacterial Species | AmpC | blaCTX-M | Int1 |
|------------------|------|----------|------|
|                  | MOX  | ACC      |      |
| A. caviae        | +    | -        | -    |
| P. penneri       | -    | -        | +    |
| A. hydrophila    | -    | -        | +    |
| K. pneumoniae    | -    | +        | -    |
| P. vulgaris      | -    | -        | -    |
| E. aerogenes     | -    | -        | -    |
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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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