Diversity and antibiotic resistance patterns of enterobacteria isolated from seafood in Thailand

Neelawan Pongsilp\textsuperscript{a} and Pongrawee Nimnoi\textsuperscript{b}

\textsuperscript{a}Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand; \textsuperscript{b}Department of Microbiology, Faculty of Liberal Arts and Science, Kasetsart University, Nakhon Pathom, Thailand

\textbf{ABSTRACT}

Contamination with enterobacteria was detectable in 89% of seafood samples from three central seafood markets in Thailand. The average numbers obtained from the same type of seafood were between 1.3 ± 0.9 and 4.5 ± 1.3 log CFU/g per sample. Eighty-one strains and 16 species were distinguished based on ERIC-PCR patterns and TP-RAPD patterns, respectively. The highest prevalence (90% of strains) was resistant to penicillin G whereas none was resistant to gentamycin. In addition, 63% exhibited multidrug resistance. The 16S rDNA sequences of a representative strain from each species exhibited 99% identity to either one of six genera including \textit{Citrobacter}, \textit{Enterobacter}, \textit{Klebsiella}, \textit{Providencia}, \textit{Serratia}, and \textit{Yersinia}. Three \(\beta\)-lactamase genes including \textit{blaTEM}, \textit{ampC}, and \textit{shv} were detected at the frequencies of 43%, 27%, and 24%, respectively. The representative strains possessing \(\beta\)-lactamase genes exhibited \(\beta\)-lactamase activity ranging from 1.96 ± 0.88 to 11.3 ± 0.37 \(\mu\)mol of hydrolyzed nitrocefin/min/mg protein.

\textbf{INTRODUCTION}

Seafood is a nutrient-rich part of a healthful diet containing a unique dietary source of the marine \(n\)-3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid, vitamin D, vitamin B12, iodine, and selenium (Dahl, Bjerrka-Jaer, Graff, Kjellved, & Klemensen, 2006; Iwamoto, Ayers, Mahon, & Swerdlow, 2010). Seafood consumption has been shown to be associated with potential health attributes including cognitive development of infant during pregnancy (Oken et al., 2005), neurologic development during gestation and infancy (Hibbeln et al., 2007), and reduction in risk of heart disease (Mozaffarian & Rimm, 2006). Nevertheless, seafood consumption is not risk-free because seafood contributes to an important proportion of food-borne illnesses and outbreaks worldwide. Among the Food and Drug Administration (FDA)-regulated food categories, seafood was responsible for the second most outbreaks and the most relative rate of illness during 2004–2013 (Center for Science in the Public Interest (CSPI), 2015). Bacteria were reported to be a major cause (54%) of food-borne disease outbreaks in the United States in 2015 (Center for Disease Control and Prevention (CDC), 2017). Food poisoning caused by enterobacteria has become a public health concern in Thailand. In 2015, 200.22 food poisoning cases per 100,000 population were reported and \textit{Salmonella} spp. were the one most frequently found (48%) among pathogenic bacteria identified from 0.57% of all patients (Bureau of Epidemiology, Thailand, 2015).

Enterobacteria, which belong to the family \textit{Enterobacteriaceae}, are known as important seafood-associated pathogens. Up to date, some members have been assigned to novel families: \textit{Budviciaceae}, \textit{Erwiniacae}, \textit{Hafniaceae}, \textit{Morganellaceae}, \textit{Pectobacteriaceae}, and \textit{Yersiniaceae} which were proposed by Adeolu, Aminj, Naushad, and Gupta (2016). Enterobacteria have been implicated in the pathogenesis of host diseases such as nonalcoholic steatohepatitis, allergy, and inflammatory bowel disease (Miyata et al., 2011). Previous reports demonstrated the presence of enterobacteria in a variety of seafood of various origins (Guo et al.,...
Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) fingerprinting of enterobacterial isolates

ERIC-PCR was performed to analyze genotypic diversity and relatedness among enterobacterial isolates as well as to distinguish individual strains. Genomic DNA of each isolate was used as a template in PCR reactions using a pair of primers ERIC2 and ERIC1R as described previously (Ogutcu, Adiguzel, Gulluce, Karadayi, & Sahin, 2009). The presence and size of the amplified fragments were determined by agarose (1% in Tris-borate-EDTA (TBE) buffer) gel electrophoresis and the unweighted pair groups using mathematical averages (UPGMA) dendrogram was constructed using the Phoretix ID Pro. software (TotalLab Ltd., Newcastle upon Tyne, UK). Enterobacterial strains with individual ERIC-PCR patterns were selected for subsequent studies.

Physiological and biochemical characteristics of enterobacterial strains

Enterobacterial strains were examined for their physiological and biochemical characteristics including 1) temperatures, pH values, and NaCl concentrations for growth; 2) production of enzymes (catalase, amylase, urease, caseinase, and protease); 3) fermentation of sugars (glucose, lactose, and sucrose); 4) decarboxylation of amino acids (arginine, lysine, and ornithine); and 5) indole, methyl red (MR), Voges-Proskauer (VP), citrate (IMViC) test.

Examination on antibiotic resistance of enterobacterial strains

Enterobacterial strains were examined for resistance to ten antibiotics as described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2012).

Detection of antibiotic resistance genes in enterobacterial strains

Genomic DNA of each strain was used as a template in PCR reactions to detect the presence of eight antibiotic resistance genes as previously described. These genes included ampC (Hanson et al., 1999), blaCTX (Mohaddam, Beidokhti, Jamehdar, & Ghahraman, 2014), blaTEM (Bert, Bramger, & Lambert-Zochovsky, 2002), blaz (Olsen, Christensen, & Aarestrup, 2006), mecA (Durun, Ozer, Duran, Onlen, & Demir, 2012), oxa1 (Onyang, Ndeda, Wandlli, Wawire, & Ochieng, 2014), oxa9 (Hanson et al., 1999), and shv (Fang, Ataker, Hedin, & Dornbusch, 2008).

Two-primers random amplified polymorphic DNA (TP-RAPD) fingerprinting of enterobacterial strains

TP-RAPD was performed to distinguish enterobacterial species. Genomic DNA of each strain was used as a template in PCR reactions using a pair of primers 8F and 1522R as described by Rivas, Velazquez, Valverde, Mateos, and Martinez-Molina (2001). The presence and size of the amplified fragments were determined by agarose (1% in TBE buffer) gel electrophoresis.

Sequence analysis of partial 16S rDNA of enterobacteria

Partial 16S rDNA of a representative strain from each TP-RAPD pattern was amplified using a pair of universal primers UN16S 926f and UN16S 1392r (Lane, 1991). PCR reactions were carried out as described by Pongsilp, Teamroong, Nuttagij, Boonkerd, and Sadowsky (2002) and the PCR...
products were purified using QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). The purified PCR products were sequenced by Bio Basic Canada Inc. (Markham, Ontario, Canada). The nucleotide sequences were aligned with reference 16S rDNA sequences using the BLASTN program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the closest genera.

**Sequence analysis of antibiotic resistance genes of enterobacteria**

To reinforce the presence of antibiotic resistance genes in seafood-associated enterobacteria, the detected antibiotic resistance genes of a representative strain from each species were amplified. The PCR products were purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). The purified PCR products were sequenced by Bio Basic Canada Inc. (Markham, Ontario, Canada). The nucleotide sequences were aligned with reference sequences using the BLASTN program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Measurement of β-lactamase activity of enterobacteria**

The β-lactamase activity of a representative strain from each species which possessed β-lactam antibiotic resistance gene(s) was measured. The β-lactamase induction and β-lactam antibiotic resistance gene assay were performed as described by Sharma, Ramnani, and Virdi (2004).

**Results and discussion**

**Prevalence of enterobacteria in seafood sold in Thailand**

Enterobacteria were under the detection limit (< 1.2 log CFU/g) in four seafood samples while the remaining 31 samples contained enterobacteria that ranged from 1.3 ± 0.2 to 5.4 ± 0.1 log CFU/g. Average numbers of presumptive enterobacteria per sample of the same type of seafood sold in three central seafood markets of Thailand are shown in Table 1. Ninety-six pure isolates of enterobacteria were derived and designated by abbreviations ENTSF followed by the number (1 to 35) that indicates the order of isolation. The numbers of enterobacterial isolates was derived from the orders 16 and 28. Enterobacteria counts were tested and five profiles were obtained. The 33 and 5 strains fermented all tested sugars. Eighty-one enterobacterial strains varied in their physiological and biochemical characteristics. All strains grew at a temperature range between 20°C and 40°C. The 18 and 5 strains were able to grow at a minimum temperature of 15°C and maximum temperature of 45°C, respectively. All strains grew at a pH range between 4 and 9. The only one strain was able to grow at a minimum pH of 3. The maximum pH for growth was 11 for 20 strains. The 56 strains tolerated the maximum NaCl concentration of 7%. The 68, 9, and 1 strains produced catalase, urease, and amylase, respectively. None was positive for caseinase production. Protease activity ranging from undetectable to 14.56 ± 1.08 units/ml supernatant was measured by azocasein protease assay (Secades & Guijarro, 1999).

The strains exhibited four profiles for sugar fermentation. Sixty-eight strains fermented all tested sugars. Eight strains fermented only glucose. The only one strain fermented both glucose and lactose while four strains fermented both glucose and sucrose. Decarboxylation of arginine, lysine, and ornithine was tested and five profiles were obtained. The 33 and 5 strains decarboxylated both lysine and ornithine. Thirty strains decarboxylated both lysine and ornithine. Eleven strains were unable to decarboxylate either one of these amino acids.

| Type of seafood | Average numbers of presumptive enterobacteria per sample of the same type of seafood (log CFU/g)* |
|-----------------|---------------------------------------------------------------------------------------------------|
| Common name     | Scientific name                                                                                   |                                                                 |
| Blue swimming  | Portunus pelagicus                                                                              | 1.8 ± 1.0                                                        |
| Crab (6)        |                                                                                                   |                                                                 |
| Banana shrimp   | Fenneropenaeus murgiennsis                                                                      | 2.5 ± 1.2                                                        |
| (5)             |                                                                                                   |                                                                 |
| Splendid squid  | Loligo fomasana                                                                                  | 1.3 ± 0.9                                                        |
| (4)             |                                                                                                   |                                                                 |
| Spotted babylon | Babylonia areolata                                                                              | 2.8 ± 0.9                                                        |
| (3)             |                                                                                                   |                                                                 |
| Scallop (3)     | Agaponeurus purpuratus                                                                            | 2.6 ± 1.5                                                        |
| Barramundi (2)  | Lates calcarifer                                                                                 | 4.5 ± 1.3                                                        |
| Bigfin reef     | Sepioteuthis lessoniana                                                                          | 2.5 ± 1.8                                                        |
| Squid (2)       |                                                                                                   |                                                                 |
| Short-bodied    | Rastrelliger brachysoma                                                                          | 3.0 ± 1.9                                                        |
| mackerel (2)    |                                                                                                   |                                                                 |
| Silver pomfret  | Pampus argenteus                                                                                 | 2.0 ± 0.4                                                        |
| (2)             |                                                                                                   |                                                                 |
| Kuruma prawn    | Marsupenaeus japonicus                                                                          | under the detection limit (< 1.2)                                 |
| (1)             |                                                                                                   |                                                                 |
| Blood cockle    | Tegillarca granosa                                                                              | under the detection limit (< 1.2)                                 |
| (1)             |                                                                                                   |                                                                 |
| Mangrove crab   | Scylla serrata                                                                                  | 3.6 ± 0.4                                                        |
| (1)             |                                                                                                   |                                                                 |
| Cuttle fish     | Sepia officinalis                                                                               | 2.7 ± 0.1                                                        |
| (1)             |                                                                                                   |                                                                 |
| John’s snapper  | Lutjanus johnii                                                                                 | 2.9 ± 0.5                                                        |
| (1)             |                                                                                                   |                                                                 |
| Green tiger     | Peneaus semisculus                                                                              | 1.8 ± 0.2                                                        |
| prawn (1)       |                                                                                                   |                                                                 |

*In cases that there were more than one sample of the same type of seafood, the values shown are the means of all samples assayed ± standard deviations. In cases that there was only one sample of the same type of seafood, the values shown are the means of three replicates ± standard deviations.

**Physiological and biochemical characteristics of enterobacterial strains**

Eighty-one enterobacterial strains varied in their physiological and biochemical characteristics. All strains grew at a temperature range between 20°C and 40°C. The 18 and 5 strains were able to grow at a minimum temperature of 15°C and maximum temperature of 45°C, respectively. All strains grew at a pH range between 4 and 9. The only one strain was able to grow at a minimum pH of 3. The maximum pH for growth was 11 for 20 strains. The 56 strains tolerated the maximum NaCl concentration of 7%. The 68, 9, and 1 strains produced catalase, urease, and amylase, respectively. None was positive for caseinase production. Protease activity ranging from undetectable to 14.56 ± 1.08 units/ml supernatant was measured by azocasein protease assay (Secades & Guijarro, 1999).

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The strains exhibited eight IMViC profiles including – + – – (56 strains), + + – + (nine strains), + + – – (five strains), – + – + (five strains), – + + – (three strains), – – + + (one strain), – – + – (one strain), and – + + + (one strain). The 14, 79, 6, and 16 strains were positive for indole production, MR reaction, VP reaction, and citrate utilization, respectively.

**Prevalence of antibiotic resistance among enterobacterial strains**

Twenty-four antibiotic resistance patterns, as shown in **Table 2**, were observed among 81 enterobacterial strains. The most common resistance pattern (25% of strains) was the ampicillin, erythromycin, penicillin G, and vancomycin co-resistance. The resistance to penicillin G, vancomycin, erythromycin, ampicillin, tetracycline, chloramphenicol, streptomycin, neomycin, and kanamycin was found in 73 (90% of strains), 62 (77%), 56 (69%), 50 (62%), 18 (22%), 3 (4%), 3 (4%), 2 (2%), and 1 (1%) strains, respectively, while none was resistant to gentamycin. The only one strain was susceptible to all ten antibiotics while the remaining strains exhibited resistance to at least one but up to six of the tested antibiotics. These ten antibiotics are sorted into six categories including 1) aminoglycosides (e.g. gentamycin, kanamycin, neomycin, and streptomycin); 2) glycopeptides (e.g. vancomycin); 3) macrolides (e.g. erythromycin); 4) penicillins (e.g. ampicillin and penicillin); 5) phenicols (e.g. chloramphenicol), and 6) tetracyclines (e.g. tetracycline). These antibiotics have been selected to determine the resistance of *Enterobacteriaceae* members in previous reports. The high prevalence of enterobacterial isolates displayed resistance to ampicillin, erythromycin, penicillin, and vancomycin. In con-

**Table 2. Antibiotic resistance patterns of seafood-associated enterobacterial strains and numbers of strain(s) belonging to each pattern.**

| Antibiotic resistance pattern | Number of resistant strain(s) (%) | Antibiotic resistance pattern | Number of resistant strain(s) (%) | Antibiotic resistance pattern | Number of resistant strain(s) (%) |
|-------------------------------|----------------------------------|-------------------------------|----------------------------------|-------------------------------|----------------------------------|
| none                          | 1 (1%)                           | AMP ERY PEN VAN               | 2 (3%)                           | ERY NEO PEN VAN               | 1 (1%)                           |
| PEN                           | 2 (3%)                           | AMP PEN TET VAN               | 2 (3%)                           | ERY PEN TET VAN               | 1 (1%)                           |
| VAN                           | 3 (4%)                           | AMP PEN TET VAN               | 6 (7%)                           | AMP CHL ERY VAN PEN VAN       | 1 (1%)                           |
| AMP PEN                       | 8 (10%)                          | ERY PEN TET VAN               | 1 (1%)                           | AMP ERY PEN TET VAN           | 5 (6%)                           |
| AMP ERY PEN                   | 1 (1%)                           | ERY PEN TET VAN               | 13 (16%)                         | CHL ERY PEN TET VAN           | 1 (1%)                           |
| ERY PEN                       | 2 (3%)                           | ERY PEN TET VAN               | 2 (3%)                           | AMP CHL ERY PEN TET VAN       | 1 (1%)                           |
| NEO PEN                       | 1 (1%)                           | AMP ERY PEN TET VAN           | 1 (1%)                           | AMP ERY KAN PEN TET VAN       | 1 (1%)                           |
| PEN VAN                       | 2 (3%)                           | AMP ERY PEN TET VAN           | 20 (25%)                         | AMP ERY PEN STR TET VAN       | 3 (4%)                           |

AMP: ampicillin (10 µg); CHL: chloramphenicol (30 µg); ERY: erythromycin (15 µg); KAN: kanamycin (30 µg); NEO: neomycin (30 µg); PEN: penicillin G (10 units); STR: streptomycin (10 µg); TET: tetracycline (30 µg); VAN: vancomycin (30 µg)

Figure 1. Dendrogram constructed from ERIC-PCR patterns of 96 enterobacterial isolates.

Figura 1. Dendrograma construido de patrones ERIC-PCR de 96 asilados enterobacterianos.
In relation to the result of antibiotic resistance, numbers of strains (s) closely related to each genus that exhibited resistance were obtained. The closest genera of 16 enterobacterial species were Citrobacter, Enterobacter, Klebsiella, Providencia, Serratia, and Yersinia with 99% identity. These sequences can be retrieved from the GenBank database under accession numbers MF593860 to MF593875. Taken together with TP-RAPD patterns, the data suggest that the seafood-associated enterobacteria included six species closely related to members of Klebsiella (34 strains), four species closely related to members of Enterobacter (30 strains), three species closely related to members of Citrobacter (three strains), one species closely related to members of Providencia (11 strains), one species closely related to members of Yersinia (two strains), and one species closely related to members of Serratia (one strain). The frequencies of the strains closely related to Klebsiella, Enterobacter, Providencia, Citrobacter, Yersinia, and Serratia in 35 seafood samples were 57%, 57%, 23%, 9%, 6%, and 3%, respectively. TP-RAPD patterns of species closely related to members of Klebsiella, Enterobacter, Citrobacter, Providencia, Yersinia, and Serratia correspond to lanes 1 to 6, 7 to 10, 11 to 13, 14, 15, and 16, in Figure 2, respectively.

In order to confirm the resistance to the antibiotic test, numbers of strains (s) closely related to each genus that showed high and low prevalences were reported for gentamycin-resistant isolates (Citron, Tyrrell, Merriam, & Goldstein, 2012; Hu, Liu, Zhang, Feng, & Zong, 2017; Kilonzo-Nthengen, Rotich, & Nahashon, 2013; Kumar, 2016). Multidrug resistant (MDR) is defined as nonsusceptibility to at least one agent in three or more antimicrobial categories (Basak, Singh, & Rajurkar, 2016). Therefore, 63% of strains were multiresistant. The previous study also noted the incidence of multiresistant enterobacteria present in seafood (Janecko et al., 2016; Nawaz et al., 2012). Antibiotic resistance may be directly introduced into seafood-associated enterobacteria via terrestrial run-off, in which antibiotic-resistant bacteria or antibiotic compounds were present. Mutidrug resistance of enterobacteria is a challenge for the global public health agenda. Enterobacteria have an intriguing ability to acquire multi-resistance in a single step by capturing several resistance genes from a variety of bacterial species and transferring genes to the same plasmids (Partridge, 2015).
strains were resistant to either one of three aminoglycosides including kanamycin, neomycin, and streptomycin.

In relation to their biochemical characteristics, shared characteristics among six species closely related to *Klebsiella* included 1) fermentation of glucose, lactose, and sucrose; 2) arginine decarboxylase-negative and lysine decarboxylase-positive reactions; 3) negative indole test; and 4) positive MR test. Similar features among four species closely related to *Enterobacter* were 1) fermentation of glucose; 2) ornithine decarboxylase-positive reaction; and 3) negative indole test. Three species closely related to *Citrobacter* shared features including 1) fermentation of glucose and lactose; 2) lysine decarboxylase-negative and ornithine decarboxylase-positive reactions; 3) positive MR test and negative VP test; and 4) negative urease test. A single species closely related to *Providencia* fermented glucose but did not ferment lactose. They were negative for arginine, lysine, and ornithine decarboxylases, positive for indole and MR tests, negative for VP test as well as displayed variable reactions for sucrose fermentation, citrate utilization, and urease production. A single species closely related to *Yersinia* fermented glucose, lactose, and sucrose. They were positive for ornithine decarboxylase and MR test but negative for arginine and lysine decarboxylase, indole test, VP test, citrate utilization, and urease production. A strain closely related to *Serratia* fermented glucose, lactose, and sucrose. It was positive for lysine and ornithine decarboxylases and MR test but negative for arginine decarboxylase, indole test, VP test, citrate utilization, and urease production. The results of carbohydrate fermentation were in accord with the phenotypic features of identified genera described in Walker, Mahon, Lehman, & Manuselis (2015). Glucose fermentation is a common characteristic of enterobacteria which is employed as a basis for their detection. Lactose fermentation is a common characteristic in most members of *Citrobacter, Enterobacter, Klebsiella, and Serratia,* but it is very rare in *Providencia.* Sucrose fermentation is common in most members of *Klebsiella* and *Serratia.* It is widely variable (0% to 100%) among species of *Enterobacter* and *Yersinia.*

### Table 3.
Numbers of strain(s) closely related to each genus that exhibited resistance to each antibiotic.

| Genus     | AMP (%) | CHL (%) | ERY (%) | GEN (%) | KAN (%) | NEO (%) | PEN (%) | STR (%) | TET (%) | VAN (%) |
|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| *Klebsiella* (n = 34) | 24 (71%) | 0 (53%) | 18 (61%) | 0 (3%) | 0 (3%) | 1 (3%) | 32 (94%) | 0 (24%) | 8 (65%) | 22 (65%) |
| *Enterobacter* (n = 30) | 20 (67%) | 2 (7%) | 24 (80%) | 0 (3%) | 1 (3%) | 0 (3%) | 28 (93%) | 2 (7%) | 6 (20%) | 23 (77%) |
| *Providencia* (n = 11) | 4 (36%) | 1 (9%) | 8 (73%) | 0 (3%) | 0 (3%) | 0 (3%) | 7 (64%) | 0 (27%) | 3 (100%) | 11 (100%) |
| *Citrobacter* (n = 3) | 1 (33%) | 0 (0%) | 3 (100%) | 0 (3%) | 0 (3%) | 0 (3%) | 1 (100%) | 1 (33%) | 1 (33%) | 3 (100%) |
| *Yersinia* (n = 2) | 1 (50%) | 0 (0%) | 2 (100%) | 0 (3%) | 0 (3%) | 0 (3%) | 2 (100%) | 0 (0%) | 0 (0%) | 2 (100%) |
| *Serratia* (n = 1) | 0 (0%) | 0 (0%) | 1 (100%) | 0 (3%) | 0 (3%) | 0 (3%) | 1 (100%) | 0 (0%) | 0 (0%) | 1 (100%) |

**AMP:** ampicilina (10 µg); **CHL:** chloramphenicol (30 µg); **ERY:** erythromycin (15 µg); **GEN:** gentamycin (10 µg); **KAN:** kanamycin (30 µg); **NEO:** neomycin (30 µg); **PEN:** penicillin G (10 units); **STR:** streptomycin (10 µg); **TET:** tetracycline (30 µg); **VAN:** vancomycin (30 µg)

**The values with the same letter are not significantly different.**

**The values shown are the means of three replicates ± standard deviations.**

### Table 4.
β-lactamase activity of the selected enterobacterial strains harboring different β-lactamase genes.

| Strain harboring β-lactamase gene(s) | Closest genus | Detected β-lactamase gene(s) | β-lactamase activity (µmol of hydrolyzed nitrocefin/min/mg protein) |
|-------------------------------------|---------------|--------------------------------|--------------------------------|
| ENTSSF 4–1                          | Enterobacter   | blaTEM                         | 3.59 ± 0.27**                  |
| ENTSSF 6–3                          | Enterobacter   | blaTEM                         | 4.09 ± 0.96                    |
| ENTSSF 1–1                          | Klebsiella     | blaTEM                         | 1.96 ± 0.88                    |
| ENTSSF 31–2                         | Klebsiella     | blaTEM                         | 4.18 ± 0.16                    |
| ENTSSF 22–1                         | Providencia    | blaTEM                         | 11.3 ± 0.37                   |
| ENTSSF 7–2                          | Serratia       | blaTEM                         | 3.2 ± 0.12                    |
| ENTSSF 23–1                         | Enterobacter   | ampicilina blatem             | 7.7 ± 0.5                      |
| ENTSSF 22–2                         | Providencia    | ampicilina blatem             | 9.73 ± 0.49                   |
| ENTSSF 8–2                          | Enterobacter   | ampicilina blatem shv         | 8.77 ± 0.38                   |
| ENTSSF 18–1                         | Providencia    | ampicilina blatem shv         | 2.59 ± 0.74                   |

**The values shown are the means of three replicates ± standard deviations.**

**The values with the same letter are not significantly different.**

**Los valores presentados corresponden a las medias de tres repeticiones ± desviación estándar.**

**Los valores que figuran con la misma letra no son significativamente diferentes.**

### Table 3.
Mediciones de cepa(s) estrechamente relacionadas con cada género que exhibió resistencia a cada uno de los antibióticos.

### Table 4.
Actividad de β-lactamasa de las cepas de enterobacterias seleccionadas.
ine the β-lactamase activity. Among the ten strains examined, the strain ENTSF 22–1 displayed the highest β-lactamase activity. The β-lactamase activity of the selected enterobacterial strains is listed in Table 4. However, it is possible that β-lactamase activity was partly resulted from other β-lactamase genes that co-occur in the same strains.

**Conclusion**

The results of this study provide information on the prevalence of enterobacteria, a major group of food-borne pathogenic bacteria, in seafood sold in Thailand that would be valuable for hygienic and sanitary management. Most of the seafood samples (89%) were positive for contamination with enterobacteria, in which 63% were multiresistant. The resistance to antibiotics in five categories was exhibited by 7% of all strains. The multi-drug resistance in nonclinical strains emphasizes the risk of spread via food and environment. This issue of concern should be involved in surveillance programs.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the Silpakorn University Research and Development Institute under Grant SURI-D 57/01/27.

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