The Thai Journal of Veterinary Medicine

Volume 51
Issue 4 *December, 2021*

Article 14

12-1-2021

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Recommended Citation

Morita, Yukio; Thwe, Su Myo; Morita, Satoshi; and Boonmar, Sumalee (2021) "Bacterial counts and prevalence of Salmonella and extended-spectrum β-lactamase producing bacteria in prawn and shrimp imported into Japan," *The Thai Journal of Veterinary Medicine*: Vol. 51: Iss. 4, Article 14.
DOI: https://doi.org/10.56808/2985-1130.3173
Available at: https://digital.car.chula.ac.th/tjvm/vol51/iss4/14

Original Article

Bacterial counts and prevalence of *Salmonella* and extended-spectrum β-lactamase producing bacteria in prawn and shrimp imported into Japan

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Abstract

The prevalence of Non-typhoidal *Salmonella* (NTS) and extended-spectrum β-lactamase (ESBL) producing bacteria was assessed in samples of imported prawn and shrimp obtained from retail markets in Japan. The overall prevalence of NTS and ESBL producing bacteria was 4%, and 14%, respectively. We identified 4 NTS isolates, including *S. enterica* subspecies *salamae*, and 3 serovars of *S. enterica* subspecies *enterica*, including Weltevreden, Abony, and Stanley. ESBL producing bacteria were isolated from 14 of 100 samples, thirteen of which harbored *Stenotrophomonas maltophilia* and that harbored *Chryseobacterium indologenes*. Among the *S. maltophilia*-positive samples, six had been imported from India, five samples came from Indonesia, one sample was from Thailand, and 1 was from Vietnam. These 14 ESBL isolates were susceptible to moxalactam, chloramphenicol, nalidixic acid, ofloxacin, and sulfamethoxazole-trimethoprim; however, all isolates were resistant to carbapenems. These findings indicate that to increase food safety, information regarding contamination by foodborne pathogens including *Salmonella* should be provided as feedback to the food's country of origin. Additionally, more detailed studies regarding the epidemiological significance of *S. maltophilia* and *C. indologenes* contamination of imported food are needed for both food-exporting and -importing countries.

Keywords: *Chryseobacterium indologenes*, Extended-spectrum β-lactamase producing bacteria, Imported prawn, Non-typhoidal *Salmonella*, *Stenotrophomonas maltophilia*

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Received May 7, 2021
Accepted August 29, 2021
https://doi.org/10.14456/tjvm.2021.89

Introduction

Uncooked meat, such as chicken, pork, beef, and ready-to-eat meats can be contaminated by bacteria including multidrug-resistant bacteria and those that cause food poisoning (Adzitey *et al*., 2021; Bushnell *et al*., 2013; Vipham *et al*., 2012). In many countries in Asia, high-pathogen avian influenza (HPAI), foot and mouth disease (FMD), classical swine fever(CSF), and African swine fever(ASF) have occurred. Uncooked meat cannot be exported from the countries where outbreaks occur to other countries, including Japan. However, large amounts of seafood, especially prawns, are imported into Japan from countries with some degree of foodborne pathogens, such as India, Indonesia, Vietnam, Myanmar, and Thailand.

Non-typhoidal *Salmonella* (NTS) is widely distributed in domestic and wild animals. Human salmonellosis caused by NTS is generally contacted through the consumption of contaminated food of animal origin, such as poultry, pork, beef, and contaminated raw eggs (Ferrari *et al*, 2019; Gal-Mor *et al*., 2014). Seafood and plants (fruits and vegetables) can also serve as vehicles of the NTS (Bergar *et al*, 2010; Ferrari *et al*, 2019). Asai *et al*. (2008) reported that 2 of 47 frozen black tiger prawns imported from Indonesia and Vietnam were positive for *S.* Weltevreden and that *salmonella* counts in positive 2 samples were 40 most-probable-number (MPN)/100g, and <30 MPN/100g, respectively. NTS contamination in seafood has also been reported, and the infections related to seafood tend to appear as sporadic outbreaks (Asai *et al*., 2008; Holtby *et al*., 2006).

Based on the food sanitation law, in Japan, all food poisoning cases found by medical doctors are investigated epidemiologically by food hygienists working on local public health center. According to annual reports between 2016 and 2020 of the Ministry of Health, Labor and Welfare, Japan, 138 NTS food poisoning cases and 3,846 patients have been reported. Many NTS cases are consumption of egg, meat and these products, however very rare case of *S.* Weltevreden and consumption of seafoods exist, in Japan.

In Japan, monitoring of antimicrobial resistance bacteria from farm animal, and from patients in hospital were performing by the Japanese veterinary antimicrobial resistance monitoring system (JVARM) from 1999, and Japan nosocomial infections surveillance (JANIS) from 2000, respectively. Existing extended-spectrum β-lactamase (ESBL) producing bacteria are a worldwide problem (Okeke *et al*., 2005), and southern, southeastern Asian countries, and Japan have a high prevalence of ESBL producing bacteria in humans and food animals (Castanheira *et al*., 2011, Kawamura *et al*., 2017, Yamamoto *et al*., 2017). In Vietnam, 72.7% of shrimp having ESBL- producing *E. coli* at a local market (Le *et al*., 2015). In India, 71.6% of 475 *E. coli* isolated from 50 fresh seafood samples (37 fish and 13 shellfish) sold in retail markets were ESBL-producing *E. coli* (Singh *et al*., 2020). There is high prevalence of ESBL-producing *E. coli* in fresh seafood including shrimp at retail market in Vietnam and India. Although countries including India, Indonesia, Thailand, Vietnam export large quantities of seafood to many countries, there seem to be no reports on the prevalence of ESBL producing bacteria in frozen prawn and shrimp in Japan.

The objective of this study, therefore, was to determine the prevalence of NTS and ESBL producing bacteria in prawn and shrimp imported into Japan.

Materials and Methods

***Sample collection and laboratory handling*:** Between November 2011 and October 2012, a total of 100 prawn and shrimp samples (45 black tiger prawns, 40 white shrimp, and 15 other shrimp) with shells were purchased from retail shops in the Tokyo area (Table 1). The samples, packed in plastic containers or bags, were kept under low temperature on ice, in refrigerators, or in freezers in the shops and were transferred to our laboratories in a box at 1–4 °C. The refrigerated samples were kept in the refrigerator at 3–5 °C, and analyzed within 24 h of collection, and the frozen samples were defrosted in refrigerator at 3–5 °C, and analyzed within 36 h of collection.

Table 1 Information about prawn and shrimp samples

Product	No. of samples	Country of origin (No. of samples)
Black tiger prawn	45	India (12), Indonesia (25), Thailand (3), Vietnam (4), and Myanmar (1)
White shrimp	40	India (20), Indonesia (12), Thailand (6), and Vietnam (2)
Unknown shrimp	15	India (8), Indonesia (2), Thailand (3), and Myanmar (2)
Total	100	India (40), Indonesia (39), Thailand (12), Vietnam (6), and Myanmar (3)

***NTS isolation methods and antimicrobial susceptibility tests*:** Briefly, for isolation of NTS, each 25-g shrimp meat sample was placed in 225 mL of buffered peptone water (Oxoid, Hampshire, UK), thoroughly mixed, and incubated at 37 °C for 18 h. Then, 1 mL or 0.1 mL of this pre-enrichment culture was added to 10 mL of Tetrathionate (TT) Broth (Oxoid) or Rappaport Vassiliadis (RV) Broth (Oxoid), respectively, and incubated at 42 °C for one day. After incubation, TT and RV cultures were streaked onto deoxycholate-hydrogen-sulfide-lactose (DHL) agar (Nissui, Tokyo, Japan) and Brilliance™ Salmonella Agar (Oxoid) and incubated at 37 °C for 18 h. Typical *Salmonella* colonies (n = 1–3) were selected from each specimen for confirmation based on biochemical characteristics (Ewing, 1986). Serotyping of *Salmonella*

enterica subsp. *enterica* isolates was performed by agglutination testing with antisera for somatic O and phase 1 and phase 2 flagellar antigens (Denka, Tokyo, Japan) according to the Kauffman–White Scheme (Grimont and Weill, 2007).

Antimicrobial susceptibility testing was performed using the disk diffusion method from the Clinical and Laboratory Standard Institute (CLSI) documents M100-S21(CLSI, 2007) and M45-A2(CLSI, 2010) using BD Sensidiscs (BD, Franklin Lakes, NJ) with Mueller–Hinton agar plates (BD). Nine antimicrobial agents on disks were used for susceptibility testing of the isolates. Individual antimicrobial agent concentrations were as follows: 10 µg ampicillin (AMP), 30 µg cefmetazole (CMZ), 10 µg streptomycin (S), 30 µg tetracycline (TE), 30 µg chloramphenicol (C), 30 µg fosfomycin (FOM), 30 µg nalidixic acid (NA), 5 µg ofloxacin (OFX), and 23.75 µg sulfamethoxazole with 1.25 µg trimethoprim (SXT). *Escherichia coli* ATCC 25922 was used as the control strain.

***Aerobic bacteria counts*:** Samples (10 g) in 90 ml of phosphate buffered saline (PBS; pH 7.2) were homogenized gently by hand for approximately 2 min. The homogenate was diluted 10^0- to 10^4-fold in PBS. A portion (50 µl) of the PBS dilution of each sample was inoculated onto plate count agar (Nissui) using a spiral plating system (Eddy Jet Spiral Plater; Iul Instruments, Barcelona, Spain). The inoculated agar plates were incubated at 35 °C for 18 h, and then colonies were counted using a semi-automated colony counting system (aCOLyte, Synbiosis, Cambridge, UK).

***Statistical analysis*:** We performed analysis of variance using f-test. Then, independent two-sample t-tests with equal or unequal variance were used to compare the aerobic bacteria counts between samples of black tiger prawns, white shrimp, and unknown shrimp, and between NTS-positive samples and the negative samples by Excel software (Excel 365 MSO, Microsoft, USA). Ingredient was only "shrimp" on food label, we classified as"unknown shrimp". Differences were considered significant if *P* values were below 0.05.

Isolation methods of ESBL producing bacteria, antimicrobial susceptibility tests, and identification methods: Samples (10 g) in 90 ml of phosphate buffered saline (PBS; pH 7.2) were gently homogenized by hand for approximately 2 min. A portion (50 µl) of the homogenate for each sample was inoculated onto ESBL agar (bioMérieux, Marcy l'Etoile, France) and KPC agar (Chromagar, Paris, France) using a spiral plating system (Eddy Jet Spiral Plater). The KPC agar is for detection of Gram-negative bacteria with a reduced susceptibility to most of the carbapenem agents. The inoculated agar plates were incubated at 37 °C for 18–24 h, and all growing colonies were picked up to use in antimicrobial susceptibility tests.

First, antimicrobial susceptibility tests were performed using nine antimicrobial agents in the form of disks in the same manner as for the *Salmonella* antimicrobial susceptibility test. When the strains were resistant to penicillins (AMP) and cephems (CMZ), a second antimicrobial susceptibility test was performed in the same manner. Thirteen antimicrobial agents alone or in combination with a beta β-lactamase inhibitor on the disks were used for susceptibility testing of the isolates. The disks contained the following amounts of antimicrobials: 30 µg moxalactam (MOX), 25 µg cefpodoxime (CPD), 30 µg cefotaxime (CTX), 30 µg ceftazidime (CAZ), 30 µg aztreonam (AZM), 30 µg ceftriaxone (CTRX), 30 µg cefpirome (CPR), 10 µg imipenem (IPM), 10 µg doripenem (DRPM), 10 µg meropenem (MEPM), 30 µg cefotaxime with 10 µg clavulanic acid (CTX/CLA), 30 µg ceftazidime with 10 µg clavulanic acid (CAZ/CLA), and 10 µg ampicillin with 10 µg sulbactam (ABPC/SBT).

ESBL producing bacilli were identified based on disk diffusion tests for *E. coli*, *Klebsiella pneumoniae*, and *K. oxytoca*, performed as described in CLSI document M100-S25 (CLSI, 2015). The diameters of the growth-inhibitory zones for CPD, CAZ, AZM, CTX, and CTRX disks were ≤17 mm, ≤22 mm, ≤27 mm, ≤27, and ≤25 mm, respectively. Next, the diameter of the growth-inhibitory zone for CAZ/CLA is more than 5 mm greater than that of CAZ. ESBL producing bacteria were identified using a commercial identification kit (ID test NF-18, Nissui).

Results

NTS prevalence in samples and antimicrobial susceptibility of isolates: As shown in Table 2, 4 (4%) of 100 of prawn and shrimp samples tested were *Salmonella* positive; 1 strain was identified as *S. enterica* subspecies *salamae* and the other 3 strains as *S. enterica* subspecies *enterica*. Subspecies *salamae* was isolated from the unknown shrimp imported from Indonesia. Serovar *S.* Weltevreden was isolated from black tiger prawns from Indonesia, *S.* Abony was isolated from white shrimp from India, and *S.* Stanley was isolated from an unknown shrimp species from India. The 4 strains of *Salmonella* isolated in this study were sensitive to 12 of the tested antimicrobial agents.

Aerobic bacteria count in commercial prawn and shrimp: The counts of aerobic bacteria per sample are shown in Table 3. The prawn and shrimp samples tested in this study showed high levels of contamination. The geometric mean of our samples was 4.8 log CFU/g, and aerobic bacteria counts from white shrimp (5.1 log CFU/g) were significantly higher than those from either black tiger prawn (4.6 log CFU/g) or unknown shrimp (4.5 log CFU/g). Aerobic bacteria counts from 4 NTS-positive samples ranged from 4.8 to 6.1 log CFU/g; the total bacteria counts in these 4 positive samples (5.4 log CFU/g) were higher than the 96 negative samples (4.8 log CFU/g).

***ESBL producing bacteria prevalence in samples examined*:** No ESBL producing *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca,* or *Acinetobacter baumannii* were isolated from these samples of imported prawn and shrimp. The antimicrobial resistance of ESBL producing bacteria isolated from our samples is shown in Table 4. ESBL producing bacilli were isolated from 14 of 100 samples, including 13 samples that harbored *Stenotrophomonas maltophilia* and 1 that harbored

Chryseobacterium indologenes. Six *S. maltophilia*-positive samples were imported from India (4 white shrimp and 2 unknown shrimp), five positive samples were imported from Indonesia (2 samples of white shrimp, twe samples of black tiger prawn, and 1 sample of an unknown shrimp species), one positive sample of white shrimp came from Thailand, and 1 sample of black tiger prawn came from Vietnam. *C. indologenes* was isolated from a sample of an unknown shrimp species imported from India. These 14 ESBL isolates were susceptible to MOX, C, NA, OFX, and SXT, but all isolates were resistant to carbapenems including IPM, DRPM, and MEPM.

Table 2 *Salmonella*-positive samples

No.	Sample	Origin	*Salmonella* subsp. or serotype	Aerobic bacteria count (log CFU/g)[a]
1	Unknown shrimp	Indonesia	*S. salamae* [b]	6.1
2	Black tiger prawn	Indonesia	*S*. Weltevreden [c]	4.9
3	White shrimp	India	*S*. Abony [c]	5.6
4	Unknown shrimp	India	*S*. Stanley [c]	4.8

[a] Total aerobic bacteria counts in the 4 positive samples (5.4 log CFU/g) were higher than the 96 negative samples (4.8 log CFU/g), statistically ($P<0.05$).
[b] *Salmonella enterica* subspecies *salamae*
[c] *Salmonella enterica* subspecies *enterica*

Table 3 Aerobic bacteria counts in commercial prawn and shrimp

Sample	No. of Samples	Aerobic bacteria count (log CFU/g)			
		Mean	SD [a]	Maximum	Minimum
Black tiger prawn	45	4.6 [b]	1.0	5.7	2.6
White shrimp	40	5.1 [b]	0.8	7.1	3.0
Unknown shrimp	15	4.5 [b]	0.8	6.1	2.6
Total	100	4.8	0.9	7.1	2.6

[a] Standard deviation [b] $P<0.05$

Discussion

The prawn and shrimp samples obtained for analysis in this study originated from south and south-east Asian countries. Four prawn or shrimp samples were found to be NTS-positive on the basis of culture methods. The mean aerobic bacteria counts in the 4 NTS-positive samples were higher than the 96 NTS-negative samples. Prawn and shrimp cultured in other Asian countries are typically imported into Japan in frozen packages, so the stage at which NTS contamination is most likely to occur is during aquaculture of these species on farms. However, contamination during processing prior to freezing is also possible. It is very important that handling of the prawn and shrimp during transportation and storage should follow proper sanitary procedures. Farming, processing, transportation, and storage of the prawn and shrimp are likely to be independent relative risk factors for contamination. Therefore, an analysis of the entire chain of handling is necessary to ensure the safety of seafood products. Asai *et al*. (2008) reported that 2 of 47 (4.3%: one sample from Indonesia, and 1 from Vietnam) black tiger prawn samples were positive for *S. enterica* subspecies *enterica* serotype Weltevreden. The results of the present study revealed a similar prevalence of NTS-positive prawns and shrimp (4.0%; 2 from Indonesia and 2 from India). Though our examined samples size is only 100 samples, the results of these studies indicate that the NTS prevalence in samples of prawn and shrimp imported to Japan from south and south-eastern Asian countries can be expected to be approximately 4%.

Among NTS isolated from seafood imported into the United States, *S*. Weltevreden was found to be the most predominant (Ponce *et al*., 2008). *S*. Weltevreden is an emerging cause of diarrheal and invasive disease in humans residing in tropical regions (Makendi *et al*, 2016). Though it is a Vietnam report, ten of 48 shrimp farms contained *S*. Weltevreden, and the isolates were closely related genetically by pulsed field gel electrophoresis analysis (Noor *et al*., 2015). About *Salmonella enterica* subsp. *salamae*, *S*. Abony and *S*. Stanley, the knowledge of relationship between those subspecies or serovar of *Salmonella* and seafood had not been reported. But the NTS also has virulence to human. NTS, especially *S*. Weltevreden control seems to be very important at shrimp farm located in tropical countries.

Table 4 The diameter of growth-inhibitory zone of antimicrobial agents and antimicrobial agents combined with beta-lactamase inhibitor tested on ESBL bacteria

Antibiotic and synthetic antibacterials [a]		Sample No. , Country of origin, Name of product, Species isolated											
		3&32	10&93	2	48	69	25	29	51	85	14	42	1
		India					Indonesia					Thailand	Vietnam
		White shrimp		Unknown shrimp		Unknown shrimp	White shrimp		Black tiger prawn		Unknown shrimp	White shrimp	Black tiger prawn
		SM [b]		SM		CI [c]	SM		SM		SM	SM	SM
Penicillins	AMP	- [d]	-	-	-	-	-	-	-	-	-	-	-
	AMP/SBT	-	-	-	-	-	-	-	-	-	-	-	-
Cephems	CMZ	-	-	-	-	-	-	-	-	-	-	-	-
	MOX	S [e]	S	S	S	S	S	S	S	S	S	S	S
	CPD	-	-	-	-	-	-	-	-	-	-	-	-
	CTX	-	-	-	-	-	-	-	-	-	-	-	-
	CTX/CLA	-	-	-	-	-	-	-	-	-	-	-	-
	CAZ	-	S	-	S	S	S	-	S	-	S	-	-
	CAZ/CLA	+ [f]	+	+	+	+	+	+	+	+	+	+	+
	AZM	-	-	-	-	-	-	-	-	-	-	-	-
	CTRX	-	-	-	-	-	-	-	-	-	-	-	-
	CPR	-	-	-	-	S	-	-	-	-	-	-	-
Carbapenems	IPM	-	-	-	-	-	-	-	-	-	-	-	-
	DRPM	-	-	-	-	-	-	-	-	-	-	-	-
	MEPM	-	-	-	-	-	-	-	-	-	-	-	-
Aminoglycosides	S	-	-	-	S	S	-	-	-	-	-	-	-
Tetracyclines	TE	-	-	-	-	-	-	-	-	-	-	-	-
Others	C	S	S	S	S	S	S	S	S	S	S	S	S
	FOM	-	-	-	-	-	-	-	-	-	-	-	-
Quinolones	NA	S	S	S	S	S	S	S	S	S	S	S	S
New quinolones	OFX	S	S	S	S	S	S	S	S	S	S	S	S
Synthetic antibacterials	SXT	S	S	S	S	S	S	S	S	S	S	S	S

[a] AMP: ampicillin; AMP/SBT: ampicillin with sulbactam; CMZ: cefmetazole; MOX: moxalactam; CPD: cefpodoxime; CTX: cefotaxime; CTX/CLA: cefotaxime with clavulanic acid; CAZ: ceftazidime; CAZ/CLA: ceftazidime with clavulanic acid; AZM: aztreonam; CTRX: ceftriaxone; CPR: cefpirome; IPM: imipenem; DRPM: doripenem; MEPM: meropenem; S: streptomycin; TE: tetracycline; C: chloramphenicol; FOM: fosfomycin; NA: nalidixic acid; OFX: ofloxacin; and SXT: sulfamethoxazole-trimethoprim; [b] *Stenotrophomonas maltophilia*; [c] *Chryseobacterium indologenes*; [d] No growth-inhibitory zone; [e] Sensitive; [f] The diameter growth-inhibitory zone of CAZ/CLA is ≥5 mm larger than that of CAZ.

NTS isolates from patients are often generally resistant to AMP, SM, and TE, which constitute some of the most widely used antibiotics in the human medical and veterinary fields in India (Singh *et al*., 2012) and Indonesia (Bukitwetan *et al*., 2007). However, the NTS isolates from prawn and shrimp in this study were sensitive to 9 of the antimicrobial agents tested. Ponce *et al* (2008) reported that NTS isolates from seafood imported into the United States showed a low frequency of antibiotic resistance. The low frequency of antibiotic resistance in NTS isolates observed in this study may be characteristic of isolates from prawn and shrimp samples in general.

In this study using only limited samples, no ESBL-producing *E. coli*, *K. pneumonia*, *K. oxytoca,* or *A. baumannii* were isolated that was directed at detecting ESBL bacterial contamination in imported prawn and shrimp. Diffusion of these ESBL producing bacteria from southern and southeastern Asian countries and nosocomial infection caused by ESBL producing bacteria are medically significant problems (Castanheira *et al*., 2011). ESBL producing bacteria identified in this study included *S. maltophilia* and *C. indologenes*. Both bacteria are ubiquitous in aqueous environments, soil, and plants and are also isolated from nosocomial sources (Berg *et al*., 1999; Chou *et al*., 2011; Zamora *et al*., 2012). In immunocompromised patients, *S. maltophilia* can lead to nosocomial infection, and to date, the clinical isolates and environmental isolates could not be adequately characterized by DNA analyses (Berg *et al*., 1999). *C. indologenes* is widely distributed in nature, and it is considered that low virulence bacteria. However, the organism is particularly among the immunocompromised patients, because of its exceptional antibiotic resistance (Mukerji *et al*., 2016). *C. indologenes* is an uncommon pathogen, and the number of cases reported has increased in recent years (Izaguirre-Anaribacorresponding and Sivapalan, 2020). Many strains of both *S. maltophilia* and *C. indologenes* have been reported to be resistant to nearly all penicillins and carbapenems. These resistant strains carry a gene on their bacterial chromosome encoding a metallo-β-lactamase enzyme (Quinn, 1998; Vartivarian *et al*., 1994; Bellais *et al*., 2000). There have been no epidemiological reports on the relationship between nosocomial infection and foodborne pathogens, including those occurring on prawn and shrimp. Prawn and shrimp are generally prepared by heating, which should inactivate NTS, *S. maltophilia,* and *C. indologenes* in prawn and shrimp. Therefore, the incidence of NTS infection and the spread of metallo-β-lactamase-producing *S. maltophilia* and *C. indologenes* can be reduced by cooking seafood. However, efforts to reduce the levels of NTS, *S. maltophilia*, and *C. indologenes* contamination in seafood should continue.

Improvement in seafood quality and safety is an important issue, and information on contamination by foodborne pathogens should be provided as feedback to the country of origin, with the aim of increasing food safety. Though, clinically important ESBL-producing organisms, such as *E. coli*, *K. pneumonia*, *K. oxytoca*, or *A. baumannii* could not isolate from prawn and shrimp in this study, monitoring of antimicrobial resistance bacteria from seafood is also important for food safety in the world. About 14% of prawn and shrimp imported from such countries were contaminated with

S. maltophilia and *C. indologenes*. Though our samples are only 100 samples, our results emphasize that detailed studies regarding the epidemiological significance of *S. maltophilia* and *C. indologenes* contamination on imported food are needed in both food-exporting and -importing countries.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science, KAKENHI, Grant-in-Aid for Scientific Research (C), Grant Number 23590756.

References

Adzitey F, Huda N, and Amir Shariff HM 2021. Phenotypic antimicrobial susceptibility of *Escherichia coli* from raw meats, ready-to-eat meats, and their related samples in one health context. Microorganisms 9: 326.

Asai Y, Kaneko M, Ohtsuka K, Morita Y, Kaneko S, Noda H, Furukawa I, Takatori K and Hara-Kudo Y 2008. *Salmonella* prevalence in seafood imported into Japan. J Food Protec. 71: 1460-1464.

Bellais S, Poirel L, Leotard S, Naas T and Nordmann P 2000. Genetic diversity of carbapenem-hydrolyzing metallo-beta-lactamases from *Chryseobacterium* (*Flavobacterium*) *indologenes*. Antimicrob Agents Chemother. 44: 3028-3034.

Berg G, Roskot N and Smalla K 1999. Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. J Clin Microbiol. 37: 3594-3600.

Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P and Frankel G 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens: fresh produce as vehicles for transmission of human pathogens. Environ Microbiol. 12: 2385–2397.

Bukitwetan P, Suryawidjaja JE, Salim OCh, Hidayat A, Herwana E and Lesmana M 2007. Serovar distribution and antibiotic susceptibility of nontyphoidal *Salmonella* isolated from pediatric patients in Jakarta, Indonesia. Southeast Asian J Trop Med Public Health 38 : 1088-1094.

Bushnell G, Mitrani-Gold F and Mundy LM 2013. Emergence of New Delhi metallo-β-lactamase type 1-producing *Enterobacteriaceae* and non-*Enterobacteriaceae*: global case detection and bacterial surveillance. Int J Infect Dis. 17:e325-333.

Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN and Mendes RE 2011. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob Agents Chemother. 55: 1274-1278.

Chou DW, Wu SL, Lee CT, Tai FT and Yu WL 2011. Clinical characteristics, antimicrobial susceptibilities, and outcomes of patients with *Chryseobacterium indologenes* bacteremia in an intensive care unit. Jpn J Infect Dis. 64: 520-524.

Clinical and Laboratory Standards Institute 2007. Performance standards for antimicrobial susceptibility testing: seventeenth informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA. USA.

Clinical and Laboratory Standards Institute 2010. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline, second edition. CLSI document M45-A2. Clinical and Laboratory Standards Institute, Wayne, PA. USA.

Clinical and Laboratory Standards Institute 2015. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.USA.

Ewing WH 1986. Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing, New York, pp. 181-318.

Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES and Conte-Junior CA 2019. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a Meta-analysis. Appl Environ Microbiol. 85:e00591-19.

Gal-Mor O, Boyle EC and Grassl GA 2014. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. Front Microbiol. 5: 391.

Grimont PAD and Weill F-X 2007. Antigenic formulae of the *Salmonella* serovars, 9th ed. Paris: Institute Pasteur. 166pp.

Holtby I, Tebbutt GM, Anwar S, Aislabie J, Bell V, Flowers W, Hedgley J and Kelly P 2006. Two separate outbreaks of *Salmonella enteritidis* phage type 14b food poisoning linked to the consumption of the same type of frozen food. Public Health 120: 817-823.

Izaguirre-Anaribacorresponding D E and Sivapalan V 2020. *Chryseobacterium indologenes*, an emerging bacteria: a case report and review of literature. Cureus 12: e6720.

Kawamura K, Nagano N, Suzuki M, Wachino J, Kimura K and Arakawa Y 2017. ESBL-producing *Escherichia coli* and its rapid rise among healthy people. Food Saf (Tokyo). 5: 122–150.

Le QP, Ueda S, Nguyen TN, Dao TV, Van Hoang TA, Tran TT, Hirai I, Nakayama T, Kawahara R, Do TH, Vien QM and Yamamoto Y 2015. Characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* in retail meats and shrimp at a local market in Vietnam. Foodborne Pathog Dis. 12:719-725.

Makendi C, Page AJ, Wren BW, Le Thi Phuong T, Clare S, Hale C, Goulding D, Klemm EJ, Pickard D, Okoro C, Hunt M, Thompson CN, Phu Huong Lan N, Tran Do Hoang N, Thwaites GE, Le Hello S, Brisabois A, Weill FX, Baker S and Dougan G 2016. A phylogenetic and phenotypic analysis of *Salmonella enterica* Serovar Weltevreden, an emerging agent of diarrheal disease in tropical regions. PLoS Negl Trop Dis. 10(2): e0004446.

Mukerji R, Kakarala R, Smith SJ and Kusz HG 2016. *Chryseobacterium indologenes*: an emerging infection in the USA. BMJ Case Rep. 2016: bcr2016214486.

Noor Uddin GM, Larsen MH, Barco L, Minh Phu T and Dalsgaard A 2015. Clonal occurrence of *Salmonella*

Weltevreden in cultured shrimp in the Mekong delta, Vietnam. PLoS One. 10:e0134252.

Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P and O'Brien TF 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis. 5: 481-493.

Ponce E, Khan AA, Cheng CM, Summage-West C and Cerniglia CE 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol. 25: 29-35.

Quinn JP 1998. Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. Clin Infect Dis. 27 Suppl 1: S117-124.

Singh AS, Nayak BB and Kumar SH 2020. High prevalence of multiple antibiotic-resistant, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in fresh seafood sold in retail markets of Mumbai, India.Vet Sci. 7:46.

Singh S, Agarwal RK, Tiwari SC and Singh H 2012. Antibiotic resistance pattern among the *Salmonella* isolated from human, animal and meat in India. Trop Anim Health Prod.44 : 665-674.

Vartivarian S, Anaissie E, Bodey G, Sprigg H and Rolston K 1994. A changing pattern of susceptibility of *Xanthomonas maltophilia* to antimicrobial agents: implications for therapy. Antimicrob Agents Chemother. 38: 624-627.

Vipham JL, Brashears MM, Loneragan GH, Echeverry A, Brooks JC, Chaney WE and Miller MF 2012. *Salmonella* and *Campylobacter* baseline in retail ground beef and whole-muscle cuts purchased during 2010 in the United States. J Food Prot. 75: 2110-2115.

Yamamoto S. Asakura H and Igimi S 2017. Recent trends for the prevalence and transmission risk of extended spectrum β-lactamases (ESBL) producing bacteria in foods. Shokuhin Eiseigaku Zasshi. 58:1-11(Japanese).

Zamora L, Fernández-Garayzábal JF, Palacios MA, Sánchez-Porro C, Svensson-Stadler LA, Domínguez L, Moore ER, Ventosa A and Vela AI 2012. *Chryseobacterium oncorhynchi* sp. nov., isolated from rainbow trout (*Oncorhynchus mykiss*). Syst Appl Microbiol. 35: 24-29.