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A survey of foodborne bacterial contamination in Chiang Mai Province, Thailand: A pilot study focused on *Aeromonas* and *Vibrio*

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ABSTRACT

Background: The consumption of raw or undercooked food contaminated with microbial pathogens is a major cause of foodborne illness. *Vibrio* and *Aeromonas* are significant pathogenic bacteria found in raw seafood. Currently, the consumption of raw and undercooked seafood, including salmon, is becoming popular in Thailand. Nevertheless, studies focusing on the prevalence of human pathogenic *Vibrio* and *Aeromonas* in seafood and salmon in Thailand are scarce.

Objective: This study aimed to detect *Vibrio* and *Aeromonas* in undercooked foods from Chiang Mai Province, Thailand.

Materials and methods: Ten food samples (five spicy mixed seafood salads and five salmon sushi) individually collected in Chiang Mai, Thailand, from August to October 2024. Samples were processed as previously described and bacteria were identified by 16S rRNA gene amplification by PCR and Sanger sequencing.

Results: The results indicated that three of ten (30%) were contaminated with human pathogenic *Vibrio*, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*. Moreover, *Aeromonas* species regarded as potential human pathogens, *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila*, were isolated from eight food samples (80%).

Conclusion: Our findings suggest that undercooked seafood in Chiang Mai, Northern Thailand, frequently contains pathogenic bacteria, posing significant food safety risks.

Introduction

Foodborne diseases are major public health concern worldwide. The primary cause of human illness is the consumption of raw or undercooked food contaminated with microbial pathogens. The World Health Organization (WHO) reported that approximately 600 million people suffer from foodborne illnesses, and 420,000 die each year.[1]

Vibrio and *Aeromonas* are gram-negative bacteria commonly found in aquatic environments, including estuaries, marine coastal water, sediments, and aquaculture settings. *Vibrio* belongs to the *Vibrionaceae* family, with 147 individual species currently identified.[2] Approximately 10 species, such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, cause human diseases. In addition, *V. parahaemolyticus* has been reported as the leading cause of foodborne infections in Asia, and is almost always associated with the consumption of raw

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fish and shellfish.[3] *Aeromonas* consists of 36 species, is currently being identified, 19 of which are potentially human pathogens including *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila.*[4]

Chiang Mai Province, a landlocked region in Northern Thailand, is located over 900 km from the coast. Salmon is imported from various salmon-producing countries. Both seafood and salmon are at risk of microbial contamination during transportation. Thus, evaluating the microbial safety of seafood and salmon in the supply chain is essential. Over the past decade, consumption of raw and undercooked seafood, including salmon, has become increasingly popular in Thailand. However, studies focusing on the prevalence of *Vibrio* and *Aeromonas* in seafood and salmon in Thailand are scarce.

The present study aimed to isolate human pathogenic *Vibrio* and *Aeromonas* from two undercooked foods; spicy mixed seafood salad and salmon sushi. Undercooked seafoods carrying potential pathogens are a concern for food safety.

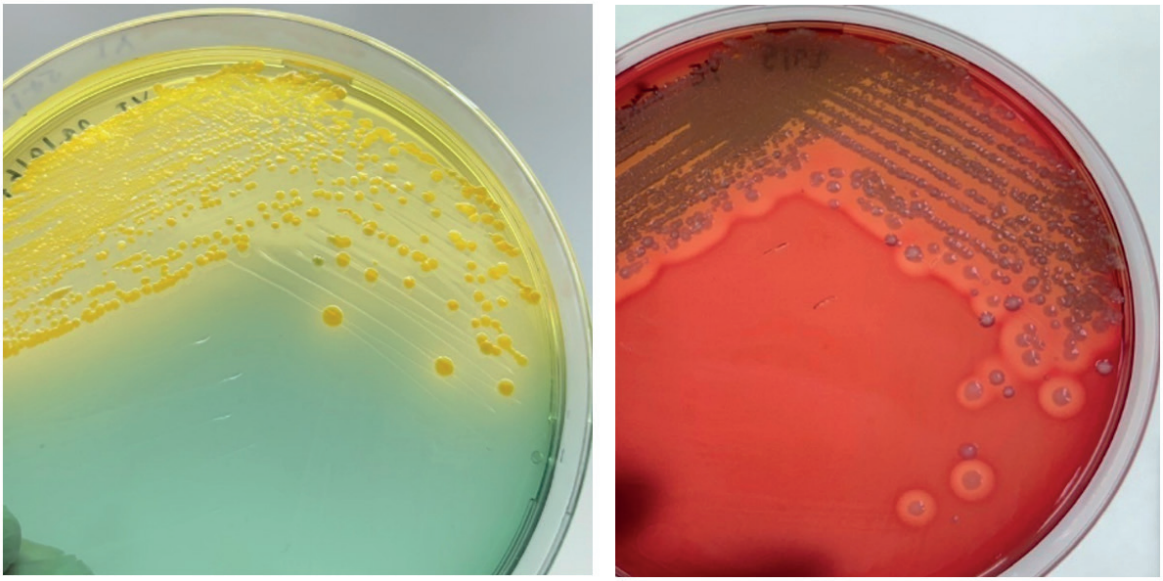
Material and methods

Sample collection

A total of 10 food samples were collected from individual restuarants in urban Chiang Mai, Thailand, between August and October 2024. These included five spicy mixed seafood salads (*Litopenaeus vannamei,Anadara granosa,Crasostrea gigas,* and *Loligo duvauceli*) and five salmon sushi.

Laboratory processing of food and bacterial isolation

Sample processing followed previous established protocols.[5] Briefly, 25 gm of material was cut with sterile scissors, transferred into a sterile blending bag containing 25 mL of alkaline peptone water (APW), and blended using a stomacher (AES MIX2 Lab blender, France) for 2 min. The solution was then aseptically transferred to a 500-ml bottle containing 200 ml of APW and incubated at 35±2 °C for 6 hrs. After incubation, 100 µl of each solution collected from the surface pellicle, was streaked on thiosulfate citrate bile salt sucrose (TBCS) agar (Himedia, USA) for *Vibrio* and blood agar supplemented with ampicillin (BASA) for *Aeromonas.*[6] Plates were incubated under suitable conditions at 35±2 °C for 24 hrs. The example of bacterial colonies that grow on TCBS and BASA plate were shown in figure 1. Colonies on TCBS plates and BASA were selected and, subsequently cultured in biochemical test media, triple sugar iron (TSI) agar slant, and motile-indole-lysine (MIL), and further incubated at 35±2 °C for 24 hrs. Suspected *Vibrio* and *Aeromonas* colonies on the TSI slant were suspended in 500 µl sterile distilled water, heated at 100 °C for 10 min, and centrifuged at 14,000 rpm for 10 min. The supernatant containing genomic DNA was stored at -20 °C until PCR amplification.



(A) (B)

Figure 1*. The example of bacterial colonies that grow. A: on TCBS, B: BASA plate.*

PCR amplification and Sanger sequencing

The combination of 16S rRNA gene amplification by conventional PCR and Sanger sequencing were used for bacterial identification in this study. The 27F (5'- AGA GTT TGA TCM TGG CTC AG -3') and 800R (5'-GAC TAC CAG GGT ATC TAA TCC-3') specific for the V1-V4 region of the 16S rRNA gene were used as universal primers for amplification.[7] The 25-µL PCR reaction contained 2× Quick Taq HS dye master mix (Toyobo, Japan), 1 pmol of 27F and 800R primers and 2 µl (approximately 100 ng) of DNA template. PCR was performed in a thermal cycler (Labcycler gradient SensoQuest, Germany) and the amplification profiles were as follows: pre-denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 30 sec, and 68 °C for 60 sec, with a final extension at 68 °C for 5 min. The expected PCR product was approximately 800 bp. PCR products were electrophoresed on a 1.5% agarose gel stained with RedSafe nucleic acid staining solution

(iNtRON Biotechnology, Inc., Korea) and visualized using a UV transilluminator (G:BOXchemi XRQ gel doc system, UK). The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Germany). Nucleotide sequences were obtained using Sanger sequencing that provided by the company (BigDye Terminator v3.1 Cycle sequencing kit, Bionics, Korea).

Identification of bacteria

Bacterial identification in this study was performed based on sequence comparison with public database. Each sequencing result was aligned with public sequences in the GenBank database using the BLASTN program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The parameters were automatically provided by the program. The highest percentage identity was selected for bacterial identification. Moreover, the evolutionary relationship among microorganisms found in this study and retrieved from the database was demonstrated using the phylogenetic tree public software (iTOL interactive, http://itol.embl.de/index.shtml).

Results

Ten food samples were included and 63 colonies suspected *Vibrio* and *Aeromonas* were selected for this study. After PCR and agarose gel electrophoresis, the results indicated that the amplified products of approximately 800-bp in size were obtained (Figure 2).

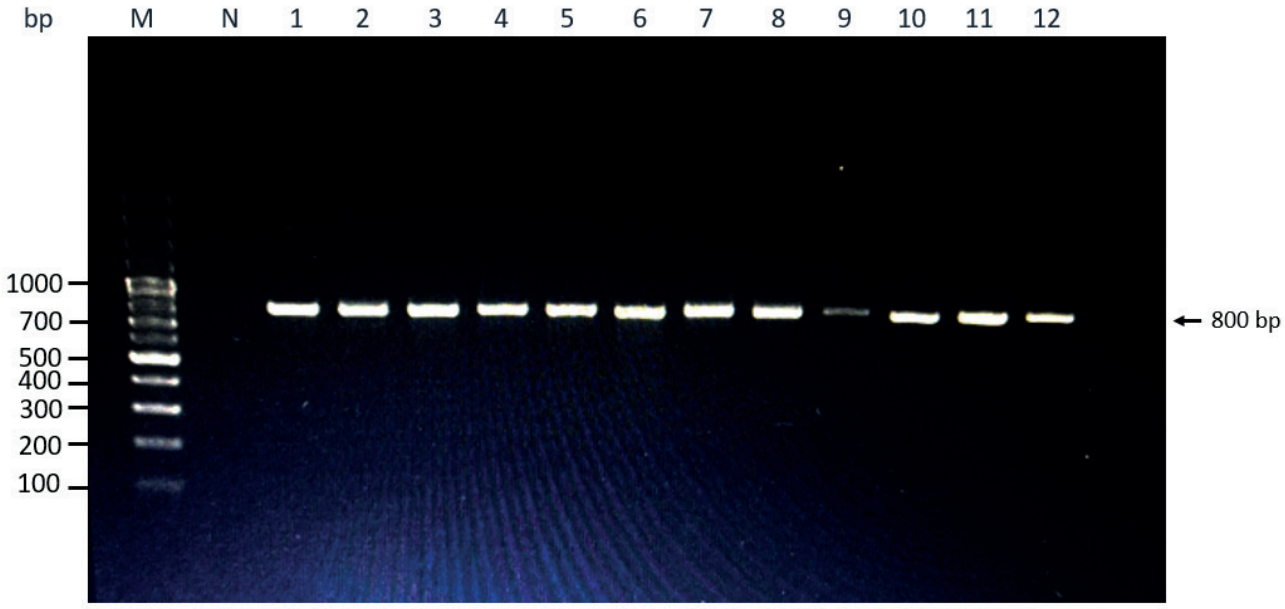


Figure 2*. The agarose gel electrophoresis of 16S rRNA gene amplification by PCR. Bacterial genomic DNA collected by the heat lysis method was used a target for 16S rRNA gene amplification by PCR and analyzed by 1.5% agarose gel electrophoresis. Approximately 800-bp amplified products were purified and sequenced using Sanger sequencing. Lane M: standard 100 bp DNA marker, lane N: negative control, lane 1-12: sample No. 34-45, respectively.*

After PCR product purification and sequencing, each bacterial species was identified using the BLASTN public program with the highest percentage identity (99-100%). The results showed that *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*, were found in three out of ten (30%) seafood samples. A mixture of *V. parahaemolyticus* and *V. vulnificus* was found in one sample. Additionally, *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila*, were isolated from eight food samples (80%). A mixture of *A. hydrophila* and *A. caviae* was found in a single sushi sample. A summary of the human pathogenic *Vibrio* and *Aeromonas* strains isolated from food is shown in Table 1. The phylogenetic analysis representing the evolutionary relationship of the 16S rRNA gene among the six- sequencing results derived from bacterial colonies in this study compared to the 23 sequences of *Aeromonas* spp. and *Vibrio* spp. retrieved from the GenBank database is illustrated in Figure 3. The 16S rRNA partial sequences of 12 isolates of *A. caviae* (isolate No. 1), *A. salmonicida* (isolate No. 63), *A. veronii* (isolate No. 49), *E. cloacae* (isolate No. 24), *E. hormaechei* (isolate No. 31), *K. pneumoniae* (isolate No. 23), *P. mirabilis* (isolate No. 6), *P. rettgeri* (isolate No. 20), *V. harveyi* (isolate No. 10), *V. mediterranei* (isolate No. 56), *V. parahaemolyticus* (isolate No. 46) and *V. vulnificus* (isolate No. 43) were submitted to GenBank under accession No. PQ855910-PQ855921, respectively.

Table 1. Summary of human pathogenic *Vibrio* and *Aeromonas* isolated from food in this study.

Microorganisms	Mixed seafood salad (N=5)	Salmon sushi (N=5)	Total (N=10)
Vibrio spp.	3*	0	3
V. parahaemolyticus	2		
V. cholerae	1		
V. vulnificus	1		
Aeromonas spp.	4	4#	8
A. caviae	1	2	
A. hydrophila	2	1	
A. veronii	1	1	
A. dhakensis		1	

***Note**: *Both of V. parahaemolyticus and V. vulnificus were isolated in one sample, #Both of A. hydrophila and A. caviae were isolated in one sample.*

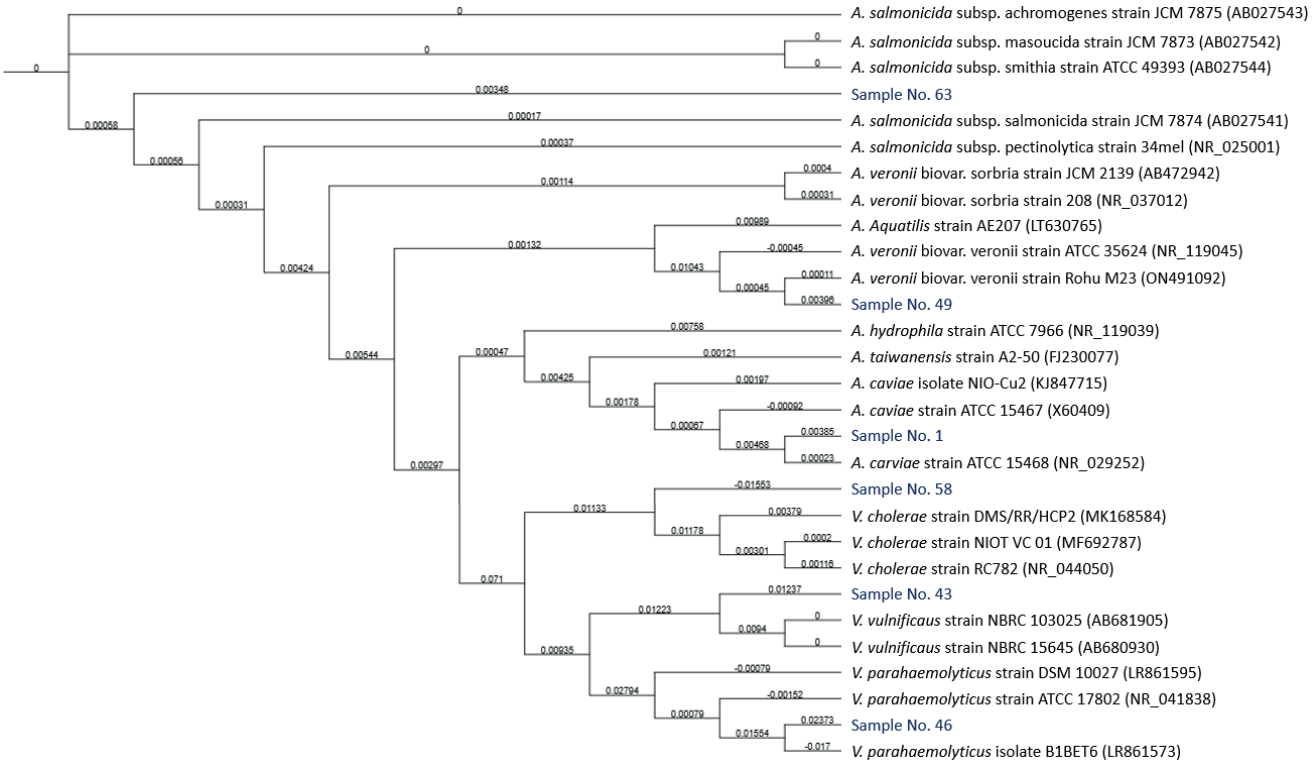


***Figure 3**. Phylogenetic analysis representing the relationship of the 16S rRNA gene among 6 sequencing results. The 16S rRNA gene of each bacterium was sequenced and identified using the BLASTN program with the highest percentage identity (99-100%). A phylogenetic tree was constructed using iTOL public software (http://itol.embl.de/itol.cgi). Samples 1, 43, 46, 49, 58, and 63 were identified as A. caviae (Accession No. PQ855910), V. vulnificus (Accession No. PQ855921), V. parahaemolyticus (accession no. PQ855920), A. veronii (accession no. PQ855912), V. cholerae (accession no. MW165382), and A. salmonicida (accession no. PQ855911), respectively. The accession numbers of the sequences retrieved from the GenBank database are shown in the blankets.*

Discussions

Foodborne illnesses remain a persistent public health challenge, often linked to the consumption of raw or undercooked food contaminated with pathogenic bacteria. In seafood, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus* are the three most common pathogens associated with human infection. *Vibrio parahaemolyticus* is recognized as a common cause of acute gastroenteritis worldwide,[8] and accounted for approximately 20-30% of bacterial foodborne cases.[9] *Vibrio cholerae* is a pathogen that causes cholera. The disease remains a serious problem in the developing world, including in Asian and African countries. *Vibrio vulnificus* is an opportunistic human pathogen that causes severe wound infections, gastroenteritis, and septicemia. A part of *Vibrio*, the *Aeromonas* is another causative agent of variety of human diseases such as gastroenteritis, hepatobiliary infection, wound infection and septicemia.[10] In particular, gastrointestinal infection is the most prevalent form, ranging in severity from mild self-limiting watery diarrhea to severe chronic infection, and antibiotics for treatment are required.[11] Lobatón et al. revealed that up to 40% of *Aeromonas*-induced gastrointestinal infections require antibiotics for treatment.[12] At least six mesophilic species have been established as human pathogens, including *A. hydrophila*, *A. caviae*, *A. veronii* biotype veronii, *A. veronii* biotype sobria, *A. schubertii*, and *A. jandaei.*[13] Recently, *A. dhakensis* has been recognized as an important human pathogen. Although the role of gastroenteritis remains controversial, *A. dhakensis* bacteremia is more lethal than bacteremia caused by other *Aeromonas* species.[14]

Of the food samples, three *Vibrio* spp., *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*, which are strongly associated with human diseases were detected in three of ten seafood samples (Table 1). *Vibrio parahaemolyticus* and *V. cholerae* were identified in two (20%) and one (10%) food samples, respectively. Similarly, the prevalence of *V. parahaemolyticus* in raw seafood isolated from local wet markets and supermarkets in Phayao Province, Northern Thailand, was studied, and approximately 22% were positive [15]. In addition, the prevalence of *V. cholerae* in seafood collected from retail markets in Yala Province, Southern Thailand, has been reported to be 34%.[16] *Vibrio mimicus* was detected in one seafood sample in this study. *Vibrio mimicus* has previously been isolated from ornamental fish in various countries, including Singapore, China, and Thailand.[17] Although rare, this microorganism has been reported to produce cholera-like toxin and can infect humans, leading to sporadic cases of diarrhea and gastroenteritis [18].

Aeromonas caviae, *A. dhakensis*, *A. veronii*, *A. hydrophila*, and *Aeromonas* spp. associated with human pathogens, were isolated from eight food samples (80%) in this study. The detection of human pathogenic *Aeromonas* spp. in edible raw salmon has never been reported in Thailand. Uncooked seafood samples from Bangkok were previously examined and approximately 27% consisting of *A. caviae*, *A. veronii* biovar sobria, and *A. hydrophila* had detectable [3]. Although pathogenic *Vibrio* spp. was not detected in salmon sushi in this study, up to 12% of salmon meat collected from supermarkets and restaurants in China was positive for *V. parahaemolyticus.*[19]

Other *Vibrio* spp. was detected in the present study, including *V. harveyi*, *V. hyugaensis*, and *V. mediterranei*. These are generally caused by vibriosis in marine animals such as bivalve mollusks and sea cucumber. *Aeromonas salmoicida* was observed in one sample of salmon sushi. Currently, there are five subspecies, namely *salmonicida*, *smithia*, *achromogenes*, *masoucida*, and *pectinolytica*. Phylogenetic tree analysis indicated that *A. salmoicida* isolated here (No. 63) was closely related to these five subspecies (Figure 3). The bacterium is classified as a psychrophilic bacterium and has been recognized as the causative agent of furanculosis and septicemia in the salmonid family.[20] Due to global warming, the abundance of bacteria in coastal waters, including *A. salmonicida*, has adapted to grow at elevated temperatures. The number of clinical cases infected with mesophilic *A. salmonicida* infection has significantly increased in recent years. Mesophilic *A. salmonicida* isolated from human blood, skin, and eyes has been reported.[21-23] Although the pathogenesis is currently unclear, human diseases, including acute gastroenteritis and foot cellulitis, caused by *A. salmonicida* have been ascribed.[24] According to climate change, infection with mesophilic *A. salmonicida* and uncommon *Aeromonas* and *Vibrio* should be raised in health awareness in the future.

Previous studies have reported challenges in accurately identifying *Aeromonas* species using conventional biochemical methods. Genetic analysis using DNA-DNA hybridization is highly accurate and is considered the gold standard method for *Aeromonas* identification. However, they are rarely used in clinical laboratories because of the high cost and long time required for analysis.[25] Identification of *Aeromonas* based on 16S rRNA gene sequences and some other housekeeping genes such as *gyrB*, *dnaJ* and *rpoB* have successfully been reported.[26] To ensure reliable bacterial identification, combined bacterial culture and identification using 16S rRNA gene amplification and sequencing was conducted for both *Vibrio* and *Aeromonas* species identification in this study.

Finally, our data implied that undercooked seafoods from spicy mixed seafood salads and salmon sushi in Chiang Mai province, Northern Thailand, frequently harbored pathogenic bacteria caused by foodborne illness. Therefore, food safety is a concern for customers.

Conclusion

Foodborne diseases are a major public health concern worldwide. In this study, the human pathogens *Vibrio* and *Aeromonas* were often detected in spicy mixed seafood salad and salmon sushi. These findings highlight the potential food safety risks associated with seafood and salmon transportation to Chiang Mai Province, Thailand.

Limitations

This study has several limitations that should be acknowledged. First, the small sample size of only 10

food samples (five spicy mixed seafood salads and five salmon sushi) limits the generalizability of the findings. A larger sample size, collected across different seasons and locations, would provide a more comprehensive understanding of bacterial contamination patterns. Second, the study was geographically restricted to Chiang Mai, a landlocked province in Thailand. The microbial contamination levels in this region may differ from those in coastal or seafood-producing areas, highlighting the need for future studies in diverse geographic settings. Third, while bacterial species were identified using 16S rRNA sequencing, the study did not assess antibiotic susceptibility patterns, which are crucial for understanding potential antimicrobial resistance (AMR) risks. Conducting antibiotic susceptibility testing in future studies would help determine whether these foodborne pathogens pose a public health threat due to drug resistance. Additionally, the study did not differentiate whether contamination originated from the seafood itself, improper food handling, or storage conditions. Future research incorporating environmental sampling, microbial source tracking, and supply chain analysis would help identify the exact sources of contamination. Another limitation is that virulence gene and toxin detection were not performed, meaning the study could not determine whether the isolated *Vibrio* and *Aeromonas* strains could cause severe disease. Finally, while PCR-based identification is a widely used method, it has limitations in distinguishing closely related bacterial species. Complementary approaches, such as whole-genome sequencing (WGS) or multilocus sequence typing (MLST), would provide higher-resolution bacterial characterization in future research. To address these limitations, future research should focus on expanding sample sizes across multiple geographic regions, incorporating seasonal variations to better assess contamination trends. Additionally, studies should include antibiotic susceptibility testing to evaluate potential antimicrobial resistance among foodborne pathogens. Investigating the sources of contamination through environmental monitoring, supply chain assessment, and microbial source tracking will also be crucial in developing targeted food safety interventions. Furthermore, the application of advanced molecular techniques, such as WGS and MLST, will enhance the accuracy of bacterial identification and provide insights into the genetic diversity and pathogenic potential of these microorganisms. These efforts will contribute to a more comprehensive understanding of foodborne bacterial risks and support the development of improved food safety policies in Thailand and beyond. Despite these limitations, the study provides valuable preliminary data on bacterial contamination in undercooked seafood in Northern Thailand, emphasizing the importance of food safety measures.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study did not involve in human subjects and laboratory animals.

References

[1] World Health Organization (WHO). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. Geneva: WHO; 2015. Available from: https://apps.who.int/iris/handle/10665/199350 [Accessed 5 Oct 2024].

[2] Sampaio A, Silva V, Poeta P, Aonofriesei F. Vibrio spp.: life strategies, ecology, and risks in a changing environment. Diversity. 2022; 14(2): 97. doi:10.3390/d14020097.

[3] Woodring J, Srijan A, Puripunyakom P, Oransathid W, Wongstitwilairoong B, Mason C. Prevalence and antimicrobial susceptibilities of Vibrio, Salmonella, and Aeromonas isolates from various uncooked seafoods in Thailand. J Food Prot. 2012; 75(1): 41-7. doi: 10.4315/0362-028X.JFP-11-291.

[4] Fernandez-Bravo A, Figueras MJ. An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity. Microorganisms. 2020; 8(1): 129. doi: 10.3390/microorganisms8010129.

[5] Department of Medical Sciences, Thailand. Standard methods for food analysis, Volume II; 2014. Available from: http://dmsc2.dmsc.moph.go.th/webroot/BQSF/index.htm [Accessed 2 Feb 2024].

[6] Gobat PF, Jemmi T. Comparison of seven selective media for the isolation of mesophilic Aeromonas species in fish and meat. Int J Food Microbiol. 1995; 24(3): 375-84. doi:10.1016/0168-1605(94)00112-M.

[7] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, *et al*. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013; 41(1): e1. doi:10.1093/nar/gks808.

[8] Ward L, Bej A. Detection of Vibrio parahaemolyticus in shellfish by use of multiplexed real-time PCR with TaqMan fluorescent probes. Appl Environ Microbiol. 2006; 72(3): 2031-42. doi: 10.1128/AEM.72.3.2031-2042.2006.

[9] Ceccarelli D, Hasan NA, Huq A, Colwell RR. Distribution and dynamics of epidemic and pandemic Vibrio parahaemolyticus virulence factors. Front Cell Infect Microbiol. 2013; 3: 97. doi:10.3389/fcimb.2013.00097.

[10] Yuwono C, Wehrhahn MC, Liu F, Zhang L. Enteric Aeromonas infection: a common enteric bacterial infection with a novel infection pattern detected in an Australian population with gastroenteritis. Microbiol Spectr. 2023; 11(4): e00286-23. doi:10.1128/spectrum.00286-23.

[11] Zhou Y, Yu L, Nan Z, Zhang P, Kan B, Yan D, *et al*.

Taxonomy, virulence genes, and antimicrobial resistance of Aeromonas isolated from extra-intestinal and intestinal infections. BMC Infect Dis. 2019; 19(1): 158. doi:10.1186/s12879-019-3766-0.

[12] Lobatón T, Hoffman I, Vermeire S, Ferrante M, Verhaegen J, Van Assche G. Aeromonas species: an opportunistic enteropathogen in patients with inflammatory bowel diseases? A single-center cohort study. Inflamm Bowel Dis. 2015; 21(1): 71-8. doi: 10.1097/MIB.0000000000000247.

[13] Janda JM, Duffey S. Mesophilic Aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev Infect Dis. 1988; 10(5): 980-97. doi:10.1093/clinids/10.5.980.

[14] Chen P-L, Lamy B, Ko WC. Aeromonas dhakensis, an increasing recognized human pathogen. Front Microbiol. 2016; 7: 793. doi: 10.3389/fmicb.2016.00793.

[15] Siriphap A, Prapasawat W, Borthong J, Tanomsirdachchai W, Muangnapoh C, Suthienkul O, *et al*. Prevalence, virulence characteristics, and antimicrobial resistance of Vibrio parahaemolyticus isolates from raw seafood in a province in Northern Thailand. FEMS Microbiol Lett. 2024; 371: 1-9. doi: 10.1093/femsle/fnad134.

[16] Preeprem S, Aksornkird T, Nuidate T, Hajimasalaeh W, Hajiwangoh Z, Mittraparp-arthorn P. Characterization and genetic relationships of Vibrio spp. isolated from seafood in retail markets, Yala, Thailand. Trends Sci. 2023; 20(10): 5962. doi: 10.48048/tis.2023.5962.

[17] Zago V, Zambon M, Civettini M, Zaltum O, Manfrin A. Virulence-associated factors in Vibrio cholerae non-01/non-O139 and V. mimicus strains isolated in ornamental fish species. J Fish Dis. 2017; 40: 1857-68. doi: 10.1111/jfd.12659.

[18] Chitov T, Kirikaew P, Yongyune P, Ruengprapan N, Sontikun K. An incidence of large foodborne out-break associated with Vibrio mimicus. Eur J Clin Microbiol Infect Dis. 2009; 28: 421-4. doi: 10.1007/s10096-008-0639-7.

[19] Xie T, Wu G, He X, Lai Z, Xhang H, Zhao J. Prevalence and genetic diversity of Vibrio parahaemolyticus strains from salmon in Chinese markets. FEMS Microbiol Lett. 2019; 366(9): fnz103. doi: 10.1093/femsle/fnz103.

[20] Dallaire-Dufresne S, Tanaka KH, Trudel MV, Lafaille A, Charette SJ. Virulence, genomic features, and plasticity of Aeromonas salmonicida subsp. salmonicida, the causative agent of fish furunculosis. Vet Microbiol. 2014; 169: 1-7. doi: 10.1016/j.vetmic.2013.06.025.

[21] Tewari R, Dudeja M, Nandy S, Kumar Das A. Isolation of Aeromonas salmonicida from human blood sample: a case report. J Clin Diag Res. 2014; 8: 139-40. doi: 10.7860/JCDR/2014/6883.4032.

[22] Kamble R. Aeromonas salmonicida furunculosis in an adult male. Int J Curr Microbiol App Sci. 2015; 4: 59-63.

[23] Vashney A, Das M, Chaudhary P, Kumari R, Yadav K. Aeromonas salmonicida as a causative agent for postoperative endophthalmitis. Middle East Afr J Ophthalmol. 2017; 24: 213-5. doi: 10.4103/meajo.MEAJO_238_17.

[24] Vincent AT, Fernandez-Bravo A, Sanchis M, Mayayo E, Figueras MJ, Charette SJ. Investigation of the virulence and genomics of Aeromonas salmonicida strains isolated from human patients. Infect Genet Evol. 2019; 68: 1-9. doi: 10.1016/j.meegid.2018.11.019.

[25] Kitagawa D, Suzuki Y, Abe N, Ui K, Suzuki K, Yamashita T, *et al*. Comparison of MALDI-TOF mass spectrometry and rpoB gene sequencing for the identification of clinical isolates of Aeromonas spp. Heliyon. 2022; 8: e11585. doi: 10.1016/j.heliyon.2022.e11585.

[26] Kupfer M, Kuhnert P, Korczak BM, Peduzzi R, Demarta A. Genetic relationships of Aeromonas strains inferred from 16S rRNA, gyrB and rpoB gene sequences. Int J Syst Evol Microbial. 2006; 56: 2743-51. doi: 10.1099/ijs.0.63650-0.

Supplement data

Table S1. 16S rRNA sequence alignment of amplified products of bacterial colonies isolated from spicy mixed seafood salad.

Restaurant	Colony No.	BLASTN results (bacterial identification)	% gene identity	Accession No.
A	BASA 1	*Aeromonas caviae* strain DSILV26 (16S ribosomal RNA gene, partial sequence)	100	MN581908
	BASA 2	*Aeromonas* sp. strain JF8 (16S ribosomal RNA gene, partial sequence)	100	MN865804
	BASA 3	*Enterobacter cloacae* strain Uyi_28 (16S ribosomal RNA gene, partial sequence)	100	MT507221
	TCBS Y 4	*Enterobacter* sp. strain XQ53 (16S ribosomal RNA gene, partial sequence)	99	OP326122
	TCBS G 5	*Enterobacter* sp. strain P2 (16S ribosomal RNA gene, partial sequence)	99	MN400353
	TCBS G 6	*Proteus mirabilis* strain S74-1 (++)-2 (chromosome, complete genome)	100	CP053614
B	BASA 10	*Vibrio harveyi* strain B14-1 (16S ribosomal RNA gene, partial sequence)	100	MK102612
	BASA 12	*Enterobacter* sp. strain P2 (16S ribosomal RNA gene, partial sequence)	99	MN400353
	BASA 13	*Vibrio* sp. strain LQ2 (16S ribosomal RNA gene, partial sequence)	100	MN420866
	BASA 14	*Pantoea agglomerans* strain 600 (16S ribosomal RNA gene, partial sequence)	100	MT585395
	TCBS Y 15	*Vibrio hyugaensis* strain DS1807-13 (16S ribosomal RNA gene, partial sequence)	99	MT269595
	TCBS G 16	*Vibrio parahaemolyticus* strain TY-47 (16S ribosomal RNA gene, partial sequence)	100	MT505701
C	BASA 23	*Klebsiella pneumoniae* strain AP-1 (16S ribosomal RNA gene, partial sequence)	99	HQ199598
	BASA 24	*Enterobacter cloacae* strain YT58 (16S ribosomal RNA gene, partial sequence)	100	MN416258
	BASA 25	*Aeromonas* sp. strain M7 (16S ribosomal RNA gene, partial sequence)	100	OM617799
	BASA 28	*Aeromonas veronii* strain NK04 (16S ribosomal RNA gene, partial sequence)	100	KP899501
	TCBS Y 31	*Enterobacter hormaechei* subsp. *xiangfangensis* strain M1E (16S ribosomal RNA gene)	100	OP492057
	TCBS Y 32	*Pseudocitrobacter* sp. strain Cco1-MG-4 (16S ribosomal RNA gene, partial sequence)	100	OP393786
	TCBS Y 33	*Enterobacter cancerogenus* strain KNUC5011 (16S ribosomal RNA gene, partial sequence)	99	JQ682639
D	BASA 42	*Aeromonas hydrophila* strain CH-GX-NN-SC-3-2021 (16S ribosomal RNA gene, partial sequence)	99	ON203021
	BASA 43	*Vibrio vulnificus* strain TSG003 (16S ribosomal RNA gene, partial sequence)	100	KC748469
	BASA 46	*Vibrio parahaemolyticus* strain H3 (16S ribosomal RNA gene, partial sequence)	100	PP389745
	TCBS G 48	*Vibrio parahaemolyticus* strain H3 (16S ribosomal RNA gene, partial sequence)	100	PP389745
E	BASA 55	*Aeromonas hydrophila* strain A9 (16S ribosomal RNA gene, partial sequence)	100	OP109737
	TCBS Y 58	*Vibrio cholerae* strain LTW-F1 (16S ribosomal RNA gene, partial sequence)	100	MW165382
	TCBS Y56	*Vibrio mediterranei* strain PD104 (16S ribosomal RNA gene, partial sequence)	100	PP967835
	TCBS G 60	*Vibrio mimicus* strain NIOT VM 06 (16S ribosomal RNA gene, partial sequence)	100	MW578858

Table S2. 16S rRNA sequence alignment of amplified products of bacterial colonies isolated from salmon sushi.

Restaurant	Colony No.	BLASTN results (bacterial identification)	% gene identity	Accession No.
F	BASA 7	*Pseudomonas* sp. strain RL104 (16S ribosomal RNA gene, partial sequence)	100	MN234077
	BASA 8	*Aeromonas hydrophila* strain AH10 (16S ribosomal RNA gene, partial sequence)	100	MT605959
	BASA 9	*Aeromonas caviae* strain DSILV (16S ribosomal RNA gene, partial sequence)	100	MN581908
G	BASA 17	*Pseudomonas* sp. strain H374 (16S ribosomal RNA gene, partial sequence)	100	MH669304
	BASA 18	*Aeromonas dhakensis* strain NR_042155.1 (16S ribosomal RNA gene, partial sequence)	100	MH071515
	BASA 19	*Aeromonas* sp. strain MK_J3 (16S ribosomal RNA gene, partial sequence)	100	MK426653
	TCBS G20	*Providencia rettgeri* strain EGAPR (16S ribosomal RNA gene, partial sequence)	100	MH368158
H	BASA 34	*Aeromonas caviae* strain CH-GX-LZ-QJ-2-1-2021 (16S ribosomal RNA gene, partial sequence)	99	ON202885
	BASA 38	*Enterobacter huaxiensis* strain GP-SG-2 (16S ribosomal RNA gene, partial sequence)	100	MW406864
	BASA 39	*Pseudomonas monteilii* strain BFPB95 (16S ribosomal RNA gene, partial sequence)	99	EF600893
	BASA 41	*Acinetobacter pittii* strain L50 (16S ribosomal RNA gene, partial sequence)	100	OQ405779
I	BASA 49	*Aeromonas veronii* strain NK04 (16S ribosomal RNA gene, partial sequence)	100	KP899501
	BASA 50	*Aeromonas* sp. strain M7 (16S ribosomal RNA gene, partial sequence)	100	OM617799
	BASA 51	*Klebsiella michiganensis* strain 5-274 (16S ribosomal RNA gene, partial sequence)	99	PP512815
	BASA 52	*Enterobacter* sp. strain NCCP-281 gene for16S rRNA, partial sequence	100	AB641890
	BASA 53	*Pseudomonas lundensis* strain SeaQual_P_B794 (16S ribosomal RNA gene, partial sequence)	99	MT626771
J	BASA 63	*Aeromonas salmonicida* strain 349 (16S ribosomal RNA gene, partial sequence)	100	OM837138