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RESEARCH ARTICLE

Household hygiene practices and foodborne pathogen contamination in traditional fermented fish from northeastern Thailand: a One Health cross-sectional study

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ABSTRACT

Background and Aim: Traditional fermented fish (*pla-som*) is widely consumed in Northeastern Thailand and contributes substantially to household nutrition and rural livelihoods. However, production is commonly conducted at the household level under limited sanitary control, increasing the risk of foodborne pathogen contamination. From a One Health perspective, human hygiene behavior, environmental sanitation, and food safety are closely interconnected. This study aimed to assess the prevalence of major foodborne bacterial pathogens in household-produced fermented fish and to determine their associations with hygiene practices.

Materials and Methods: A cross-sectional analytical study was conducted between May and October 2020 in Kalasin Province, Northeastern Thailand. A total of 144 fermented fish samples were collected from 23 registered household production sites. Microbiological analyses were performed to detect *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. using standard bacteriological methods. Hygiene conditions were evaluated using a structured checklist based on national Good Manufacturing Practice (GMP) criteria covering six domains: location and building, equipment and utensils, production process control, sanitation, maintenance and cleaning, and personal hygiene. Binary logistic regression was used to identify associations between hygiene factors and bacterial contamination.

Results: *S. aureus* was the most prevalent contaminant (72.92%), followed by *Salmonella* spp. (57.64%), *E. coli* (40.28%), and *B. cereus* (12.50%). *E. coli* contamination was significantly associated with poor equipment and utensil hygiene (Adjusted odds ratio [AOR] = 16.61; 95% CI: 3.43–80.46; $p < 0.001$) and inadequate building conditions (AOR = 0.11; 95% CI: 0.02–0.50; $p = 0.005$). *S. aureus* contamination was strongly linked to substandard sanitation (AOR = 0.15; 95% CI: 0.06–0.38; $p < 0.001$) and poor personal hygiene (AOR = 12.00; 95% CI: 2.52–57.09; $p = 0.002$). *Salmonella* spp. contamination was associated with inadequate sanitation, maintenance and cleaning, and personal hygiene, whereas no hygiene-related factors were significantly associated with *B. cereus* contamination.

Conclusion: Household-level fermented fish production in Northeastern Thailand is characterized by high levels of foodborne pathogen contamination, strongly linked to modifiable hygiene practices. Strengthening GMP- and Water, sanitation, and hygiene-based hygiene training for small-scale producers—particularly targeting equipment cleanliness, sanitation, and personal hygiene—can substantially reduce contamination risks and support safer traditional food production within a One Health framework.

Keywords: food safety, fermented fish, household hygiene, One Health, *Salmonella*, *Staphylococcus aureus*, traditional food production, water sanitation.

INTRODUCTION

Foodborne diseases continue to represent a major global public health challenge, largely resulting from the consumption of foods contaminated with pathogenic microorganisms. The World Health Organization (WHO) estimates that foodborne diarrheal diseases affect approximately 600 million people annually and are responsible for about 420,000 deaths, corresponding to a loss of nearly 33 million disability-adjusted life years (DALYs), with around 90% of this burden occurring in low- and middle-income countries [1, 2]. In the United States, the Centers

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for Disease Control and Prevention reported that 31 major foodborne pathogens cause an estimated 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths each year [3]. In Europe, *Campylobacter* spp. and *Salmonella* spp. remain the most frequently reported foodborne pathogens, with 148,181 and 77,486 cases, respectively, documented in 2023 [4]. The Southeast Asia region accounts for more than 150 million cases and 175,000 deaths annually, ranking second globally after Africa. In this region, *Shigella* spp. and enterotoxigenic *Escherichia coli* are the leading causes of illness, whereas *Salmonella* typhi contributes most significantly to mortality [5]. In Thailand, food poisoning incidence has increased steadily, with morbidity rates rising from 92.1 to 137.8 per 100,000 population between 2020 and 2022 [6]. Overall, the WHO has identified more than 250 foodborne diseases, of which 20%–40% are associated with diarrheal illness linked to inadequate hygiene and poor water quality [7]. Common pathogens, including *Bacillus cereus*, *E. coli*, and *Salmonella* spp., highlight the persistent need for safe food handling, effective sanitation, and hygienic food processing practices [8, 9].

Fermented fish (*pla-som*) is a traditional food widely consumed across the Mekong region, particularly in Thailand, Laos, Vietnam, and Cambodia, where it serves as an affordable source of protein and income for rural households [10]. Fermentation enables fish preservation during periods of seasonal abundance and supports local food security. However, household-level production is often conducted under limited sanitary control, creating conditions that favor microbial contamination. From a One Health perspective, the safety of fermented fish is closely interconnected with human hygiene practices, environmental sanitation, and pathogen transmission along the food chain [11]. In northeastern Thailand, where freshwater resources are abundant and traditional fermentation practices are deeply embedded in local culture, contamination may occur at multiple stages, including raw material handling, processing, and fermentation, thereby posing potential health risks to consumers. Ensuring product safety, therefore, depends on the use of high-quality raw materials and the maintenance of hygienic conditions throughout the production process.

Microbiological contamination of fermented fish has emerged as an important food safety concern in Thailand. Pathogens frequently detected in these products include *B. cereus*, *E. coli* (notably *E. coli* O157), *Salmonella* spp., *Staphylococcus aureus*, *Vibrio cholerae*, and *Listeria monocytogenes* [12]. Several studies have reported high contamination rates: in northern Thailand, *E. coli*, *S. aureus*, and *Salmonella* spp. were detected in 73.3%–86.4% of *pla-som* samples, while 83.3% of *pla-ra* production sites failed to meet Good Manufacturing Practice (GMP) standards and were contaminated with *E. coli*, *B. cereus*, and *Clostridium perfringens* [13–15]. Comparable findings have been reported in neighboring Mekong countries. In Laos, *Salmonella* spp. were detected in 70.2% of retail meats and *B. cereus* in 90% of fermented soybean samples [16]. In Vietnam, *L. monocytogenes* and non-typhoidal *Salmonella* are commonly found in fish and seafood [17], while in Cambodia, *B. cereus* contamination in fermented fish ranged from 10^2 to 2.3×10^4 CFU/g [18].

According to the Thai Microbiological Quality Criteria for fermented fish, acceptable limits are defined as *E. coli* <3 most probable number (MPN)/g, *S. aureus* <100 CFU/g, *C. perfringens* and *B. cereus* <1,000 CFU/g, and the absence of *Salmonella* spp. in a 25 g sample [12, 19]. To mitigate contamination risks, the Ministry of Public Health enforces GMP standards encompassing six domains: production environment, equipment hygiene, process control, sanitation, maintenance, and personnel hygiene [20]. Despite these regulations, most household- and community-level producers operate outside formal GMP frameworks due to limited knowledge, infrastructure, and access to clean water. As a result, traditional production systems remain particularly vulnerable to microbial contamination, especially when products are consumed raw or under-fermented.

Consistent with findings from the poultry and aquaculture sectors, previous studies have demonstrated that poor hygiene practices are significantly associated with increased levels of bacterial contamination [13, 21], underscoring the importance of targeted hygiene interventions to improve food safety in traditional fermented food production systems.

Despite the growing body of evidence documenting microbial contamination in traditional fermented fish products in Thailand and neighboring Mekong countries, several critical gaps remain. First, most existing studies have focused primarily on prevalence and enumeration of foodborne pathogens, providing limited insight into the underlying hygiene-related determinants that drive contamination at the household production level. In particular, few investigations have systematically evaluated multiple dimensions of hygiene, including location and building conditions, equipment and utensil cleanliness, production process control, sanitation, maintenance, and personal hygiene, using standardized, GMP-based assessment tools.

Second, available studies are often conducted at market, retail, or industrial scales, whereas household and community-level producers, who dominate traditional fermented fish production in northeastern Thailand,

remain underrepresented. These small-scale operations typically function outside formal regulatory oversight, yet they play a central role in rural food security and livelihoods. The absence of robust, field-based evidence linking specific hygiene practices to pathogen contamination limits the development of targeted, context-appropriate interventions.

Third, although the One Health framework emphasizes the interconnectedness of human behavior, environmental sanitation, and foodborne disease transmission, few studies on fermented fish safety have explicitly applied this approach. The interactions among human hygiene behaviors, environmental conditions near freshwater sources, and microbial hazards in traditionally fermented foods have not been adequately characterized. Moreover, limited research has integrated observational hygiene assessments with microbiological data using multivariable analytical methods to identify independent risk factors for contamination.

Finally, there is insufficient evidence to inform practical hygiene improvement strategies tailored to household producers, particularly those aligned with national GMP standards and water, sanitation, and hygiene principles. Without such evidence, policy formulation, producer training programs, and One Health–oriented food safety interventions remain largely generic and insufficiently grounded in local production realities.

To address these gaps, the present study aimed to systematically investigate the association between hygiene practices and foodborne pathogen contamination in household-level fermented fish (*pla-som*) production in northeastern Thailand. Specifically, the study sought to determine the prevalence and microbiological quality of *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp. in fermented fish produced by registered household enterprises; to assess hygiene conditions across six GMP-based domains, location and building, equipment and utensils, production process control, sanitation, maintenance and cleaning, and personal hygiene, using a structured observational checklist; and to identify key hygiene-related risk factors associated with pathogen contamination through multivariable statistical analysis.

By integrating microbiological findings with detailed hygiene assessments, this study aimed to generate context-specific, evidence-based insights to support improved food safety management in traditional fermented fish production. The findings are intended to inform GMP- and Water, sanitation, and hygiene (WASH)-oriented interventions, strengthen hygiene training programs for household producers, and contribute to One Health–aligned food safety policies that reduce foodborne disease risks while preserving culturally important traditional food systems in northeastern Thailand and the wider Mekong region.

MATERIALS AND METHODS

Ethical approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and relevant national guidelines for research involving human participants. Ethical approval was obtained from the Human Ethics Committee of Khon Kaen University, Thailand (Approval No. HE6421).

Prior to data collection, all study objectives, procedures, potential risks, and benefits were clearly explained to the participants in the local language. Written informed consent was obtained from all participants before their enrollment in the study. Participation was entirely voluntary, and participants were informed of their right to refuse or withdraw from the study at any stage without any penalty or effect on their relationship with local authorities or institutions.

To ensure confidentiality and anonymity, no personal identifiers were recorded in the data collection tools or laboratory records. All data were coded and stored securely, accessible only to the research team, and used solely for academic and scientific purposes. Observations and interviews were conducted in a non-intrusive manner to minimize any disruption to routine production activities.

The study involved observational hygiene assessments and microbiological analysis of fermented fish products only and did not involve invasive procedures, clinical interventions, or experimentation on humans or animals. All laboratory analyses were performed in accordance with established biosafety and food microbiology guidelines. The research team adhered strictly to ethical standards of integrity, transparency, and respect for participants throughout the study.

Study period and location

This cross-sectional analytical study was conducted between May and October 2020 at 23 fermented fish production sites in Kalasin Province, northeastern Thailand. All selected sites were registered in the local enterprise database. Kalasin Province is one of Thailand's major fermented fish–producing regions and is

characterized by abundant freshwater resources that supply raw materials for fermentation and support extensive household-scale pla-som production.

Fermented fish sample size determination

The sample size for the fermented fish was calculated using G*Power (version 3.1) [22], based on an estimated contamination rate of 33.3% in raw meat [21], with a significance level of 0.05 and a statistical power of 0.84. The minimum required sample size was 136. To account for possible nonresponse or laboratory-related errors, a total of 144 fermented fish samples were collected from all 23 household production sites.

Sampling strategy and sample collection

Purposive sampling was employed to obtain fermented fish samples from registered household enterprises. Most products were prepared from cyprinoid fish, the most commonly used freshwater species in the study area. The samples included various forms of fermented fish, including whole fish and sliced products. For each product type, four samples from different production days were selected to ensure consistency in product type, production process, and timing. Samples were collected from three separate containers of each product type, with a target total weight of at least 300 g. All samples were placed in sterile plastic bags, stored in ice boxes, and transported to the laboratory for microbiological analysis within 6 h of collection.

Microbiological analysis

Microbiological analyses were performed to detect pathogenic bacteria commonly associated with gastrointestinal infections, including *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp. All laboratory procedures followed the guidelines of the Bacteriological Analytical Manual [23]. It is acknowledged that the fermented fish matrix, characterized by high salt content, low pH, and high protein levels, may suppress the recovery of stressed pathogens, thereby reducing detection sensitivity.

Sample preparation

Approximately 25 g of each fermented fish sample was aseptically transferred into sterile stomacher bags containing 225 mL of Butterfield's phosphate-buffered dilution water to obtain a 1:10 dilution. The mixture was homogenized using a stomacher at 300 rpm for 2 min. Serial dilutions (1:100, 1:1,000, and 1:10,000) were subsequently prepared using the same diluent.

Detection of *E. coli*

Presumptive phase

A three-tube MPN method was applied [24]. One milliliter of each dilution was inoculated into 9 mL of lauryl sulfate broth (LSB; Himedia, India) containing inverted Durham tubes and incubated at 35°C for 24 ± 2 h. Tubes showing gas formation were considered presumptive positive; negative tubes were reincubated for an additional 24 h.

Confirmed phase

A loopful from gas-positive LSB tubes was transferred to *E. coli* broth (Himedia) containing Durham tubes and incubated at 44.5°C for 24 ± 2 h. Gas production confirmed the presence of fecal coliforms. Tubes without gas were re-incubated and re-evaluated at 48 ± 2 h. The MPN of *E. coli* was determined using standard MPN tables.

Completed phase

Positive *E. coli* broth cultures were streaked onto eosin methylene blue agar (Himedia) and incubated at 35°C for 18–24 h. Colonies suggestive of *E. coli* appeared dark-centered, with or without a metallic sheen. Up to five colonies per plate were transferred to nutrient broth and incubated at 35°C for 18–24 h. Isolates were Gram-stained and identified as Gram-negative, non–spore-forming rods, followed by biochemical confirmation using methyl red–Voges–Proskauer, sulfide–indole–motility, and Simmons citrate tests. Isolates showing + + – – (biotype 1) or – + – – (biotype 2) IMViC patterns were identified as *E. coli*. *E. coli* American Type Culture Collection (ATCC) 25922 was used as a positive control to ensure analytical reliability.

Detection of *S. aureus*

A direct plate count method was used [25]. A 0.1 mL aliquot of the 1:10 diluted sample was spread onto duplicate Baird-Parker agar plates (Difco, USA) and incubated at 35°C–37°C for 45–48 h. Colonies appearing black to gray with clear halos were considered presumptive *S. aureus*. At least five colonies were selected for Gram

staining (Gram-positive *Staphylococci*), inoculated into brain heart infusion broth (Himedia), and incubated at 35°C–37°C for 18–24 h. Coagulase testing was performed using rabbit plasma containing EDTA (Himedia), and positive coagulation was observed over a 6-h period. *S. aureus* ATCC 29213 served as a positive control.

Detection of *B. cereus*

Detection of *B. cereus* followed the spread plate method [26]. A 0.1 mL aliquot of the 1:10 dilution was spread onto duplicate mannitol egg yolk polymyxin B agar plates (Difco, USA) and incubated at 30°C for 16–24 h. Colonies displaying a pink color with lecithinase activity were enumerated. Five representative colonies were Gram-stained, and Gram-positive bacilli with central or subterminal spores were subcultured on nutrient agar slants (Himedia) and incubated at 30°C for 24 h. Biochemical identification included nitrate reduction, acetyl methyl carbinol production using modified Voges–Proskauer medium, glucose fermentation in phenol red glucose broth under anaerobic conditions, and tyrosine degradation on tyrosine agar. *B. cereus* was used as a positive control.

Detection of *Salmonella* spp.

The detection of *Salmonella* spp. followed Thai standard procedures [27]. One milliliter of the 1:10 diluted sample was inoculated into 10 mL of selenite cysteine broth (Himedia) and incubated at 37°C for 18 ± 2 h. Enriched cultures were streaked onto duplicate xylose lysine deoxycholate (XLD) and Salmonella–Shigella (SS) agar plates (Himedia) and incubated at 35°C for 24 h. Suspected colonies, pink with or without black centers on XLD and colorless with black centers on SS, were examined by Gram staining and confirmed biochemically using triple sugar iron, lysine decarboxylase, urease, and Simmons citrate tests. *Salmonella* group B was used as a positive control.

Hygiene assessment

Hygiene assessment was conducted at all 23 fermented fish production sites in Kalasin Province, focusing on producers' hygiene practices. The hygiene standards of 23 workers were evaluated.

Data were collected through structured interviews and direct observations using an observational study design. Environmental and personal hygiene practices were assessed based on six criteria: (1) location and building (13 items; 11 points), (2) equipment and utensils (4 items; 6 points), (3) production process control (12 items; 15 points), (4) sanitation (13 items; 13 points), (5) maintenance and cleaning (3 items; 5 points), and (6) personal hygiene (9 items; 10 points).

Each criterion was scored using a three-level scale: good compliance (2 points), fair compliance (1 point), and non-compliance (0 points) [20]. A minimum overall score of 60% was required to pass, with each section also required to achieve at least 60% and without critical deficiencies, such as the use of water or food additives that did not meet Ministry of Public Health standards. The hygiene checklist was adapted from national food safety standards and pretested at five fermented fish production sites in Khon Kaen Province. The pretest yielded a Cronbach's alpha of 0.93, indicating excellent internal consistency. All observations were conducted by a single trained researcher using the same checklist across all sites to minimize inter-observer variation and ensure standardized data collection.

Statistical analysis

Descriptive statistics, including means, standard deviations, and contamination rates expressed as percentages, were used to summarize hygiene conditions and microbiological findings. Binary logistic regression analysis was performed to evaluate associations between hygiene-related factors and pathogenic microorganisms. Variables with a p-value <0.25 in univariate analysis were included in the multivariate model. Model assumptions were assessed prior to analysis. Multicollinearity was evaluated using the variance inflation factor, with values <10 considered acceptable. Model performance was assessed using the Akaike Information Criterion and Bayesian Information Criterion (BIC), and model calibration was evaluated using the Hosmer–Lemeshow goodness-of-fit test, with a p-value >0.05 indicating a good fit. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were calculated, and statistical significance was set at $p < 0.05$. All statistical analyses were performed using STATA version 18 (StataCorp LLC, College Station, TX, USA) under the license of Khon Kaen University.

RESULTS

Pathogenic bacterial contamination in fermented fish products

Table 1 summarizes the average contamination levels and prevalence of foodborne pathogens detected in pla-som samples from northeastern Thailand. *S. aureus* was the most prevalent pathogen, detected in 72.92% of

samples, followed by *Salmonella* spp. (57.64%) and *E. coli* (40.28%). In contrast, *B. cereus* showed the lowest contamination rate, occurring in only 12.50% of samples, which may reflect comparatively better control of conditions unfavorable to its growth. Overall, this contamination pattern highlights an urgent need for targeted improvements in food safety practices, particularly to reduce the high prevalence of *S. aureus* and *Salmonella* spp. in traditional fermented fish products.

Table 1: Microbiological quality and prevalence of foodborne pathogens in household-produced fermented fish (*pla-som*) samples (n = 144).

Foodborne pathogen	Mean ± SD	Unqualified samples (n)	Unqualified samples (%)	95% CI
Escherichia coli (MPN/g)	$1.73 \pm 6.22 \times 10^4$	58	40.28	32.53–48.55
Staphylococcus aureus (CFU/g)	$0.83 \pm 1.79 \times 10^4$	105	72.92	65.02–79.59
Bacillus cereus (CFU/g)	$5.53 \pm 4.20 \times 10^4$	18	12.50	7.99–19.03
Salmonella spp.	NA	83	57.64	49.36–65.50

Data are presented as mean ± SD, number and percentage of samples complying with the Thai community product standard for fermented fish. Microbiological criteria were defined as *Escherichia coli* <3 MPN/g, *Staphylococcus aureus* <100 CFU/g, *Bacillus cereus* <1,000 CFU/g, and absence of *Salmonella* spp. in 25 g of sample. Percentages were calculated based on the total number of samples analyzed (n = 144), and 95% CI are provided where applicable. MPN = most probable number, CFU = colony-forming units, SD = standard deviation, CI = confidence interval, NA = not applicable.

Hygienic status of fermented fish production sites

The assessment of six hygienic criteria across 23 fermented fish production sites in Kalasin Province revealed substantial variability in compliance (Table 2). Maintenance and cleaning exhibited the highest level of poor hygiene, with 18 sites (78.26%) failing to meet acceptable standards. This was followed by inadequate hygiene related to equipment and utensils, observed in 15 sites (65.22%). Poor compliance was also evident for location and building conditions, with 13 sites (56.52%) rated as substandard, while sanitation criteria were not met at 12 sites (52.17%).

Table 2: Hygienic quality scores and qualification status of fermented fish production sites based on GMP criteria (n = 23).

Criteria	Mean score ± SD	Not qualified (score <60), n	Not qualified (%)	Qualified (score ≥60), n	Qualified (%)
Location and building	57.45 ± 27.87	13	56.52	10	43.48
Equipment and utensils	47.92 ± 25.35	15	65.22	8	34.78
Control of production process	69.28 ± 10.82	6	26.09	17	73.91
Sanitation	55.22 ± 20.60	12	52.17	11	47.83
Maintenance and cleaning	53.19 ± 20.33	18	78.26	5	21.74
Personal hygiene	66.88 ± 13.11	5	21.74	18	78.26
All criteria	60.19 ± 14.82	21	91.30	2	8.70

Hygienic conditions were assessed using a structured Good Manufacturing Practice (GMP)–based checklist encompassing six domains: location and building, equipment and utensils, control of production process, sanitation, maintenance and cleaning, and personal hygiene. Scores are presented as mean ± standard deviation (SD). Production sites were classified as qualified when the overall score was ≥ 60 and not qualified when the score was < 60, in accordance with national food hygiene assessment criteria. Percentages were calculated based on the total number of production sites evaluated (n = 23). The "All criteria" row represents the overall hygienic performance across all assessed domains.

In contrast, production process control demonstrated relatively better compliance, with only 6 sites (26.09%) classified as having poor hygiene. Personal hygiene showed the most favorable results, with deficiencies identified in only 5 sites (21.74%). Despite these variations, overall hygiene performance remained concerning, as 21 out of 23 production sites (91.30%) failed to meet acceptable standards when all criteria were considered collectively.

Association between hygiene practices and foodborne pathogen contamination

Significant associations were identified between hygiene practices and bacterial contamination in fermented fish products. *E. coli* contamination was strongly associated with poor location and building hygiene (AOR = 0.11; 95% CI: 0.02–0.50; p = 0.005) and inadequate hygiene of equipment and utensils (AOR = 16.61; 95% CI: 3.43–80.46; p < 0.001), as presented in Table 3.

Contamination with *S. aureus* was significantly associated with three hygiene-related factors: inadequate control of the production process (AOR = 3.37; 95% CI: 1.02–11.16; p = 0.047), substandard sanitation practices (AOR = 0.15; 95% CI: 0.06–0.38; p < 0.001), and poor personal hygiene (AOR = 12.00; 95% CI: 2.52–57.09; p = 0.002) (Table 4).

No significant associations were observed between hygiene-related factors and *B. cereus* contamination at

the pla-som production sites. In contrast, *Salmonella* spp. contamination was significantly associated with inadequate sanitation practices (AOR = 0.13; 95% CI: 0.03–0.52; p = 0.004), insufficient maintenance and cleaning (AOR = 0.27; 95% CI: 0.06–0.38; p = 0.018), and poor personal hygiene (AOR = 4.70; 95% CI: 1.65–13.37; p = 0.004), as shown in Table 5.

Table 3: Association between hygienic practices and *Escherichia coli* contamination in household-produced fermented fish (*pla-som*) samples (n = 144).

Hygienic criteria	Hygienic status	Contaminated, No. (%)	Not contaminated, No. (%)	Crude OR	AOR (95% CI)	p-value
Location and building	Not qualified	31 (39.74)	47 (60.26)	0.95	0.11 (0.02–0.50)	0.005*
	Qualified	27 (40.91)	39 (59.09)			
Equipment and utensils	Not qualified	43 (48.86)	45 (51.14)	2.61	16.61 (3.43–80.46)	<0.001**
	Qualified	15 (26.79)	41 (73.21)			
Control of production process	Not qualified	16 (53.33)	14 (46.67)	1.96	2.24 (0.87–5.78)	0.10
	Qualified	42 (36.84)	72 (63.16)			
Sanitation	Not qualified	34 (50.00)	34 (50.00)	2.17	1.07 (0.31–3.76)	0.91
	Qualified	24 (31.58)	52 (68.42)			
Maintenance and cleaning	Not qualified	48 (40.68)	70 (59.32)	1.10	1.77 (0.61–5.11)	0.29
	Qualified	10 (38.46)	16 (61.54)			
Personal hygiene	Not qualified	17 (53.13)	15 (46.88)	1.96	1.04 (0.39–2.74)	0.94
	Qualified	41 (36.61)	71 (63.39)			

Data are presented as number (percentage) of samples classified as contaminated or not contaminated according to the hygienic status of production sites. Associations between hygienic criteria and *Escherichia coli* contamination were evaluated using binary logistic regression analysis. Crude odds ratios (OR) were obtained from univariate models, and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were derived from multivariable models controlling for potential confounders. Hygienic criteria were categorized as qualified (score ≥60%) or not qualified (score <60%) based on Good Manufacturing Practice (GMP) assessment standards. Statistical significance was set at p < 0.05 (*) and p < 0.001 (**).

Table 4: Association between hygienic practices and *Staphylococcus aureus* contamination in household-produced fermented fish (*pla-som*) samples (n = 144).

Hygienic criteria	Hygienic status	Contaminated, No. (%)	Not contaminated, No. (%)	Crude OR	AOR (95% CI)	p-value
Location and building	Not qualified	55 (70.51)	23 (29.49)	0.77	0.99 (0.24–4.07)	0.99
	Qualified	50 (75.76)	16 (24.24)			
Equipment and utensils	Not qualified	62 (70.45)	26 (29.55)	0.72	2.99 (0.29–30.34)	0.35
	Qualified	43 (76.79)	13 (23.21)			
Control of the production process	Not qualified	24 (80.00)	6 (20.00)	1.62	3.37 (1.02–11.16)	0.047*
	Qualified	81 (71.05)	33 (28.95)			
Sanitation	Not qualified	43 (63.24)	25 (36.76)	0.39	0.15 (0.06–0.38)	<0.001**
	Qualified	62 (81.58)	14 (18.42)			
Maintenance and cleaning	Not qualified	89 (75.42)	29 (24.58)	1.92	2.19 (0.80–5.94)	0.13
	Qualified	16 (61.54)	10 (38.46)			
Personal hygiene	Not qualified	30 (93.75)	2 (6.25)	7.40	12.00 (2.52–57.09)	0.002*
	Qualified	75 (66.96)	37 (33.04)			

Data are presented as number (percentage) of samples classified as contaminated or not contaminated according to the hygienic status of production sites. Associations between hygienic criteria and *Staphylococcus aureus* contamination were evaluated using binary logistic regression analysis. Crude odds ratios (OR) were obtained from univariate models, and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were derived from multivariable models after adjustment for potential confounding factors. Hygienic criteria were classified as qualified (score ≥60%) or not qualified (score <60%) based on Good Manufacturing Practice (GMP)–based assessment standards. Statistical significance was defined as p < 0.05 (*) and p < 0.001 (**).

Table 5: Association between hygienic practices and *Salmonella* spp. contamination in household-produced fermented fish (*pla-som*) samples (n = 144).

Hygienic criteria	Hygienic status	Contaminated, No. (%)	Not contaminated, No. (%)	Crude OR	AOR (95% CI)	p-value
Location and building	Not qualified	39 (50.00)	39 (50.00)	0.50	2.73 (0.72–10.31)	0.14
	Qualified	44 (66.67)	22 (33.33)			
Equipment and utensils	Not qualified	42 (47.73)	46 (52.27)	0.33	0.44 (0.10–2.04)	0.29
	Qualified	41 (73.21)	15 (26.79)			
Control of the production process	Not qualified	13 (43.33)	17 (56.67)	0.48	0.91 (0.30–2.83)	0.87
	Qualified	70 (61.40)	44 (38.60)			
Sanitation	Not qualified	27 (39.71)	41 (60.29)	0.24	0.13 (0.03–0.52)	0.004*
	Qualified	56 (73.68)	20 (26.32)			
Maintenance and cleaning	Not qualified	64 (54.24)	54 (45.76)	0.44	0.27 (0.09–0.80)	0.018*
	Qualified	19 (73.08)	7 (26.92)			

Hygienic criteria	Hygienic status	Contaminated, No. (%)	Not contaminated, No. (%)	Crude OR	AOR (95% CI)	p-value
Personal hygiene	Not qualified	22 (68.75)	10 (31.25)	1.84	4.70 (1.65–13.37)	0.004*
	Qualified	61 (54.46)	51 (45.54)			

Data are presented as number (percentage) of samples classified as contaminated or not contaminated according to the hygienic status of production sites. Associations between hygienic criteria and bacterial contamination were evaluated using binary logistic regression analysis. Crude odds ratios (OR) were obtained from univariate models, and adjusted odds ratios (AOR) with 95% confidence intervals (CI) were derived from multivariable models after adjustment for potential confounding factors. Hygienic criteria were classified as qualified (score ≥60%) or not qualified (score <60%) based on Good Manufacturing Practice (GMP)–based assessment standards. Statistical significance was defined as $p < 0.05$ (*) and $p < 0.001$ (**).

DISCUSSION

Overview of foodborne pathogen contamination in *Pla-som*

This study investigated the relationship between hygiene practices at household-level pla-som (fermented fish) production sites and foodborne pathogen contamination in Northeastern Thailand. A total of 144 fermented fish samples collected from 23 household production sites were analyzed for contamination with *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp. The findings showed that 59.6% of samples complied with microbiological standards for *E. coli*, whereas 40.3% exceeded acceptable limits, indicating persistent food safety challenges within traditional fermented fish production systems.

The heatmap analysis (Figure 1) demonstrated a clear association between hygiene deficiencies and pathogen prevalence. *S. aureus* was the most frequently detected pathogen and was primarily linked to poor personal hygiene and inadequate maintenance practices, suggesting that human handling was a major source of contamination. *Salmonella* spp. and *E. coli* exhibited moderate prevalence across hygiene categories, reflecting multiple contamination pathways related to environmental and process hygiene. In contrast, *B. cereus* was detected least frequently, likely due to its reduced persistence in high-salt, acidic fermentation environments. Collectively, these findings emphasize the importance of strengthening both personal and environmental hygiene to improve the safety of household pla-som production.

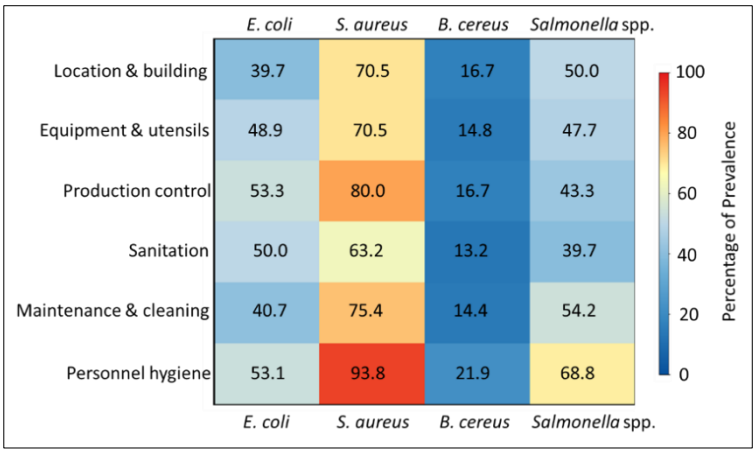

Figure 1. Heatmap showing the prevalence (%) of foodborne pathogens in household-produced fermented fish (*pla-som*) samples stratified by hygienic criteria. The heatmap displays the percentage prevalence of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. across six hygienic domains of household production sites: location and building, equipment and utensils, control of the production process, sanitation, maintenance and cleaning, and personal hygiene. Color intensity reflects the level of pathogen prevalence, with warmer colors indicating higher prevalence and cooler colors indicating lower prevalence.

Hygiene factors associated with *E. coli* contamination

Multivariate analysis identified two key hygiene-related factors significantly associated with *E. coli* contamination: deficiencies in location and building hygiene ($p = 0.005$) and inadequate hygiene of equipment and utensils ($p < 0.001$). Production sites with non-compliant equipment and utensils were 16.6 times more likely to exhibit *E. coli* contamination. As a fecal coliform bacterium widely used as an indicator of food hygiene, *E. coli* contamination reflects inadequate handwashing, improper cleaning practices, or the use of contaminated water [28]. Cross-contamination during handling, mixing, or packaging further contributes to microbial loads. Preventive measures, including the use of smooth-surfaced utensils, sealing containers after washing, and implementing integrated pest management strategies, can reduce contamination risks [29, 30]. Regular handwashing and the use of clean water for utensil cleaning remain essential components of WASH practices [31]. Given the strong associations observed, improving equipment and utensil hygiene should be prioritized in intervention programs.

Personal hygiene and *S. aureus* contamination

Significant associations were observed between *S. aureus* contamination and inadequate personal hygiene ($p = 0.002$) as well as poor production process control ($p = 0.047$). Production sites failing to meet hygiene standards had up to a 12-fold higher risk of contamination. *S. aureus* is a commensal bacterium commonly present

on human skin and in nasal passages and can be readily transferred to food through direct contact. Its halotolerance allows it to survive in salted products such as fermented fish [32]. Effective prevention relies on proper handwashing, the use of gloves during handling, and thorough equipment cleaning, all of which should be emphasized in hygiene training programs for small-scale producers.

B. cereus contamination and environmental sources

Most samples (87.5%) complied with microbiological standards for *B. cereus*, while 12.5% exceeded acceptable limits, with contamination detected at 10 production sites. *B. cereus* is a spore-forming bacterium commonly found in soil and aquatic environments and is capable of surviving in salty, protein-rich matrices [33]. Inadequate cleaning of raw fish and equipment, as well as improper temperature control during storage, are likely contributors to contamination. Effective control of *B. cereus* requires rigorous washing procedures, appropriate temperature management, and continuous quality monitoring of raw materials.

***Salmonella* contamination and sanitation deficiencies**

Salmonella spp. contamination was significantly associated with poor sanitation ($p = 0.004$), inadequate maintenance and cleaning ($p = 0.018$), and poor personal hygiene ($p = 0.004$). Inadequate sanitation increased contamination risk by nearly fivefold. Rather than being intrinsic to fish, *Salmonella* spp. are typically introduced through contaminated water or improper handling practices [34, 35]. Comparable studies in Thailand have reported *Salmonella* prevalence of 24% in beef, 57% in supermarket chicken, 53% in shrimp, and 23% in raw food samples from retail markets in Bangkok [36–38]. Such contamination is commonly linked to shared utensils for raw and cooked products, insufficient handwashing, and improper cooking or storage practices.

Comparison with findings from neighboring countries

The contamination rates observed in this study represent a significant public health concern and highlight the potential contribution of fermented fish to the regional burden of foodborne diseases measured in DALYs. Compared with neighboring Mekong countries, Thailand exhibited notably higher contamination levels. In Laos, *Salmonella* spp. were detected in 70.2% of retail meats, although data on fermented fish are lacking. No bacterial contamination in fermented fish has been reported in Vietnam, while studies in Cambodia have identified low levels of *B. cereus*, *Clostridium* spp., *S. aureus*, and *E. coli*, with *Salmonella* spp. absent in 10 fermented fish samples [16–18]. In these settings, contamination reports often focus on fermented vegetables or meats, whereas raw poultry consumption is more frequently linked to liver fluke infections [39]. The traditional consumption of raw or under-fermented pla-som further elevates public health risks, particularly when silver barb (*Barbonymus* spp.), a host of *Opisthorchis viverrini*, is used, increasing the risk of cholangiocarcinoma in the Mekong region. These combined microbial and parasitic hazards underscore the need for integrated food safety education and prevention strategies.

Food safety and the public health nexus

Although direct evidence linking *pla-som* consumption to foodborne illness remains limited, sustained exposure risk is driven by cultural dietary habits. Consumers often prefer raw or lightly fermented pla-som for its flavor, despite potential health risks. Interviews with producers revealed a tendency to prioritize production volume and speed over hygiene, with many lacking formal GMP training and perceiving gloves as impractical. These behavioral gaps highlight the need for context-specific hygiene education that clearly communicates the economic and health benefits of safe production. The coexistence of bacterial and parasitic hazards in pla-som production represents a dual public health threat, particularly given the association between *O. viverrini* infection and cholangiocarcinoma in the region [40]. Integrating microbial and parasitic control strategies through targeted health education can help mitigate these risks while preserving traditional food practices.

One Health and antimicrobial resistance (AMR) considerations

Although AMR was not assessed in this study, the detection of *E. coli*, *S. aureus*, and *Salmonella* spp. in pla-som has important implications within a One Health framework. These bacteria are frequently reported as multidrug-resistant in Southeast Asia, where traditional foods and aquaculture environments can serve as reservoirs for resistant strains. AMR *E. coli* and *Salmonella* have been detected in red tilapia and aquaculture water, suggesting potential transmission from production environments to consumers [41–43]. Future studies incorporating antimicrobial susceptibility testing would provide critical insights into AMR transmission pathways at the human–animal–environment interface. Monitoring fermented food products should therefore be integrated into Thailand's national AMR surveillance strategy.

Strengthening hygiene and WASH-based interventions

Reducing foodborne pathogen contamination in pla-som production requires coordinated interventions across the entire value chain, from aquaculture to household processing. The application of WASH and GMP principles can substantially improve food safety at relatively low cost. Essential practices include the use of clean water, regular sanitation of tools, maintenance of hygienic fermentation containers, and separation of raw and cooked utensils [44–47]. Effective control of *Salmonella* spp. and *S. aureus* requires strict sanitation, proper water management, and routine equipment disinfection. Additional measures, such as freezing fish at −20°C to inactivate *O. viverrini* metacercariae [48] and incorporating antibacterial herbs during fermentation, may further enhance safety without compromising product quality. From a One Health perspective, improving hygiene in *pla-som* production supports human health, protects aquatic ecosystems, and promotes sustainable livelihoods while reducing the circulation of AMR bacteria in community food systems.

Policy and community-level recommendations

Institutionalizing hygiene training programs for household *pla-som* producers is essential for achieving long-term improvements in food safety. Training initiatives should emphasize clean water use, safe fish selection, hand hygiene, and equipment sanitation, while integrating GMP and WASH principles. Routine microbial monitoring and AMR awareness should be incorporated into local extension services. Effective implementation of the One Health approach requires close collaboration among public health authorities, veterinary services, and environmental agencies. Integrating fermented food hygiene into Thailand's One Health Action Plan for Food Safety and Zoonoses would facilitate systematic risk monitoring and coordinated, multi-sectoral responses. Policymakers should prioritize investment in training, surveillance, and community engagement to ensure the safety and sustainability of traditional fermented food production systems.

CONCLUSION

This study demonstrated a strong and consistent association between hygiene practices at household-level *pla-som* (fermented fish) production sites and foodborne pathogen contamination in Northeastern Thailand. Among the 144 samples analyzed from 23 production sites, *S. aureus* was the most prevalent pathogen (72.92%), followed by *Salmonella* spp. (57.64%) and *E. coli* (40.28%), whereas *B. cereus* was detected less frequently (12.50%). Multivariate analysis identified critical hygiene-related risk factors, including poor equipment and utensil hygiene and inadequate building conditions for *E. coli* contamination; substandard sanitation and personal hygiene for *S. aureus* contamination; and deficiencies in sanitation, maintenance, and personal hygiene for *Salmonella* spp. contamination. These findings confirm that modifiable hygiene practices are key determinants of microbial safety in traditional fermented fish production systems.

The results have direct implications for improving food safety in household fermented fish production. Interventions should prioritize improving the cleanliness of equipment and utensils, strengthening sanitation and maintenance practices, and reinforcing personal hygiene through handwashing and protective measures during handling. Integrating GMP and WASH principles into producer training programs can substantially reduce contamination risks at a relatively low cost. Such improvements not only protect consumer health but also enhance product quality, marketability, and consumer confidence, thereby supporting rural livelihoods.

A major strength of this study lies in its integrated approach, combining microbiological testing with standardized, GMP-based hygiene assessments across multiple domains. The use of multivariate statistical analysis allowed the identification of independent hygiene-related risk factors, providing robust, context-specific evidence applicable to household-scale production systems. Additionally, the focus on traditional *pla-som* production addresses a critical yet under-researched component of food safety in the Mekong region.

Several limitations should be acknowledged. The cross-sectional design limits causal inference, and the study was confined to a single province, potentially limiting generalizability to other regions. AMR profiling and molecular characterization of isolates were not performed, limiting insight into strain diversity and resistance patterns. Moreover, seasonal variation and consumer handling practices were not assessed.

Future studies should expand to multiple regions and seasons to capture broader variability in hygiene practices and contamination patterns. Incorporating antimicrobial susceptibility testing and molecular typing would strengthen understanding of One Health risks, including AMR transmission. Intervention-based studies evaluating the effectiveness of GMP- and WASH-focused training programs are also warranted to inform evidence-based policy and practice.

In conclusion, household *pla-som* production in Northeastern Thailand is characterized by substantial foodborne pathogen contamination driven largely by preventable hygiene deficiencies. Strengthening hygiene education, improving sanitation infrastructure, and integrating traditional fermented food safety into One Health–oriented surveillance and policy frameworks are essential steps toward reducing foodborne disease risks. Addressing these challenges will help safeguard public health while preserving the cultural and economic value of traditional fermented foods in Thailand and the wider Mekong region.

DATA AVAILABILITY

The supplementary data can be made available from the corresponding author upon request.

AUTHORS' CONTRIBUTIONS

NS: Conceptualization, methodology, investigation, data collection and analysis, visualization, original draft preparation, and manuscript review and editing. RK: Supervision, conceptualization, methodology, investigation, funding acquisition, resource acquisition, original draft preparation, and manuscript review and editing. KS: Methodology and manuscript review and editing. WW: Methodology, data analysis, and manuscript review and editing. All authors have read and approved the final version of the manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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