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Assessing Prevalence and Antibiotic Resistance of *Escherichia coli* and Other Enterobacteriaceae Isolated from Cambodian Fermented Fish and Vegetables

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Abstract

Escherichia coli (*E. coli*) and other Enterobacteriaceae isolated from fermented products were evaluated for pH, salt tolerance, and multidrug resistance to nine antimicrobial categories, including acetamides, aminoglycosides, β-lactams, cephalosporins, fluoroquinolones, polymyxins, quinolones, sulfonamides, and tetracycline. *E. coli* was identified in 34% of total fermented samples, followed by *Proteus* spp. (18%), *Providencia* spp. (3%), and *Citrobacter* spp. (3%). Other minor Enterobacteriaceae species detected in 6% of the total samples were *Enterobacter aerogenes*, *Enterobacter cloacae*, *Ewardsiella tarda*, *Klebsiella oxytoca*, *Kluyvera cryocrescens*, *Morganella moganii*, *Raoultella planticola*, *Raoultella terrigena*, and *Salmonella enterica*. Most isolated bacteria can withstand 8% NaCl concentration. However, they were inhibited at 4 pH. Over 50% of the isolated *E. coli, Proteus mirabilis*, *Proteus penneri*, and *Proteus vulgaris* were multidrug-resistant to β-lactams, polypeptides cyclic, sulfonamides, and tetracyclines.

Discipline: Food
Additional key words: fermented foods, food hygiene, multidrug resistance bacteria

Introduction

Fermented foods are popular culinary products worldwide, particularly in Asia, and they are widely used in various daily cuisines, including main dishes, side dishes, and seasonings. Fermented fish are manufactured in Cambodia from small-to-medium-sized raw fish, and fermented vegetables are produced from various vegetables (Chrun et al. 2017). The fermentation process is divided into two methods: spontaneous fermentation—natural self-fermentation; culture-dependent ferments—fermented by adding starter cultures to improve product quality (Dimidi et al. 2019).

Meanwhile, the Tonle Sap Lake, Northern Cambodia, has a network of rivers suitable for natural agriculture, wherein massive supply of fresh fish and vegetables could be sourced (LeGrand et al. 2020). Depending on the source, fish and vegetables are fermented to prolong shelf-life. *Prahok* (fish paste), *Pha-ork* (fermented fish mixed with carbohydrates), *Mam* (fermented fish combined with vegetables), *Trey Praheum* (short-time fermented fish), and other fermented fish products are delicacies for Cambodians (Chuon et al. 2014). Some fermented fish, including *Prahok*, are produced with high salt content to prolong its storage life. Other traditionally fermented fish, including *Plaa-som* (Thai whole fish, 6%-11% salt), *Suan yu*, (Chinese whole fish, 3% salt), *Narezushi* (Japanese whole fish, 20%-30% salt with cooked vegetable components), and other techniques, such as mild heat treatment and high-pressure manufacturing (HPP) may be used with various types of fermented fish (Isola et al. 2022). *Chruok* is the favorite fermented vegetable meal among local foods in Cambodia. Cucumber, radish, papaya, mustard greens,

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bean sprouts, cabbages, shallots, and other vegetables are commonly used as ingredients.

Research on Thai fermented fish paste (*Pla-Ra*) showed contamination to potentially pathogenic bacteria including *Enterobacter aerogenes*, *Clostridium perfringens*, *Escherichia coli* (*E. coli*), *Listeria innocua*, and *Staphylococcus aureus* (Ananchaipattana et al. 2012, Pusporini & Pasuwan 2020). A *Salmonella* strain was isolated from 11 Thai fermented vegetables (Ananchaipattana et al. 2014). Additionally, previous studies on Cambodian fermented fish and vegetables have shown contamination to potentially pathogenic bacteria such as *E. coli*, *Cronobacter sakazakii*, *Enterobacter* spp., opportunistic non-Enterobacteriaceae, *Staphylococcus* spp., and *Listeria* spp. (Chuon et al. 2014, Chrun et al. 2017, Soeung et al. 2017).

In Cambodia, fermented fish and vegetable production mainly uses the spontaneous fermentation method, wherein pathogenic bacteria may contaminate during the process or sale in the wet market (FAO-WHO 2004). Numerous food items, including fermented food, can cause foodborne illnesses in Cambodia; the exact causes of food and foodborne hazards are unidentified in most cases (Thompson et al. 2021). Chrun et al. (2017) confirmed the possible microbiological hazard contamination in Cambodian fermented vegetables.

Antimicrobial drug-resistant bacteria, including food contaminating multidrug-resistant (MDR) bacteria, are considered a potential hazard (Doyle 2015). Drug-resistant bacteria may be discharged from farm animals and spread through the environmental waters near farms (Manyi-Loh et al. 2018). For example, drug-resistant bacteria were isolated from market purchased fish and vegetables sourced from waters of North Africa (Zekar et al. 2017). The contaminated water or manure in farm soil may transfer the drug-resistant bacteria to the crops used for fermented food production.

This study aimed to determine the prevalence of *E. coli* and other Enterobacteriaceae in fermented products purchased in Cambodia and to examine their tolerance to pH, salt concentration, and multidrug resistance. Although Enterobacteriaceae are reportedly resistant to first-line antibiotics, data on antimicrobial resistance (AMR) in Cambodia remain limited (Reed et al. 2019). In this study, screening the AMR and multidrug resistance of isolates from fermented fish and vegetables was essential.

Materials and methods

1. Sample collection

All 220 samples were randomly obtained from six provinces in Cambodia: Pursat, Kampong Chhnang, Kampong Cham, Kampong Thom, Siem Reap, Kandal, and Phnom Penh City. The fermented samples consisted of five categories: *Prahok* (n = 26), *Pha-ork* (n = 64), *Mam* (n = 23), *Trey proheum* (n = 5), and all types of fermented vegetable samples (*Chruok*, n = 102). The samples were packed in sterile zipped plastic bags and stored at low temperatures (0°C-4°C) until the experiment started.

2. Determination of the physicochemical properties of the collected samples

The samples were determined using pH, salt concentrations, and water activity (a_w). The sample was weighed at 10 g and ground; then, a volume of 10 ml distilled water was added. A calibrated electrode was then dipped into the sample, and a pH measurement was measured using a digital pH meter "LAQUA F-74" (Horiba Co. Ltd., Tokyo, Japan) (Vijayakumar & Adedeji 2017).

Titration with silver nitrate ($AgNO_3$) was used to determine the salt content. A 10 ml volume of the 10-times diluted sample was transferred to the conical flask, and 1-ml neutral potassium chromate (0.25 M) indicator was added and titrated with 0.1-M $AgNO_3$. Simultaneously, the liquid was constantly swirled until a red color appeared, and the solution remained brick red. The salt content of the original samples was determined using the acquired values as follows (Sezey & Adun 2019).

Each sample's a_w was measured without dilution at 25 ± 0.3°C using an Aqualab water activity device (Decagon Devices, Pawkit, USA).

3. Microbiological analysis of the collected samples

Twenty-five grams of sample were homogenized in 225-ml buffered peptone water (Oxoid, UK) using a lab stomacher for 2 min and incubated for 18 h-20 h at 35°C as pre-enrichment (Hoorfar & Baggesen 1998). *E. coli* was isolated on Eosin Methylene Blue agar (Oxoid, UK) and Chromogenic Coliform Agar (Oxoid, UK). Then, it was incubated for 24 h at 35°C (Sichewo et al. 2014). *Salmonella spp.* was re-enriched by transferring 0.1 ml into Rappaport-Vassiliadis broth (Oxoid, UK) and 1 ml into Tetrathionate Brilliant Green broth (Oxoid, UK), and then incubated for 24 h at 42°C. After incubation, a loopful of each broth was plated onto Salmonella and Shigella agar (Oxoid, UK) and DHL agar (Merck, Germany) and incubated at 37°C for 24 h (Mores Rall et al. 2005). Biochemical assays were conducted after the production of pure colonies. Pure presumptive cultures were streaked on Biolog Universal Growth Agar and cultured for 24 h at 33°C. Based on its phenotypic pattern in the GEN III MicroPlate, the bacterium was identified using Biolog's Microbial Identification Systems software (Al-Dhabaan & Bakhali 2017).

4. Tolerance tests for salt (NaCl) and pH

The tolerance test was slightly modified by Peristiwati et al. (2019). Tryptic soy broth (Merck, Germany) containing different salt concentrations of 4%, 6%, 8%, and 10% were prepared. The pH tolerance test was determined using hydrogen chloride (HCl) with pH (4, 5, and 6) adjustments. Then, 0.1 mL tryptic soy broth culture of the tested strains was inoculated into 10 mL salt or HCl-containing broth and incubated for 24 h at 35°C.

5. Antibiotic susceptibility tests

We chose five kinds of bacteria (*E. coli*, *P. mirabilis*, *P. penneri, P. vulgaris*, and *S. enterica*) for the assay. *E. coli* and *S. enterica* were chosen as indicator bacteria and foodborne pathogen, respectively. Other three *Proteus* strains were chosen as the typical spoilage bacteria of fermented fish.

The disk diffusion method was used according to the Clinical & Laboratory Standards Institute (CLSI 2020) using Mueller-Hinton agar (Oxoid, UK). Against the isolated bacteria, the 17 antibiotic discs' concentrations (Oxoid, USA) were used: florfenicol (FFC 30 µg), enrofloxacin (ENR 5 µg), levofloxacin (LEV 5 µg), oxytetracycline (OT 30 µg), penicillin G (P 10 µg), amoxicillin (AML 10 µg), gentamicin (CN 10 µg), ampicillin (AMP 10 µg), tetracycline (TE 30 µg), ciprofloxacin (CIP 5 µg), colistin sulfate (CT 10 µg), sulfamethoxazole (SXT 25 µg), cephazolin (KZ 30 µg), norfloxacin (NOR 10 µg), oxacillin (OX 1 µg), cefoxitin (FOX 30 µg), and ceftazidime (CAZ 30 µg). According to the European Centre for Disease Prevention and Control (Magiorakos et al. 2012), the criteria for defining MDR, extensively drug-resistant (XDR), and pan-drug-resistant (PDR) in Enterobacteriaceae are as follows: multi-drug resistance (MDR) non-susceptible to ≥1 agent in ≥3 antimicrobial categories; XDR: non-susceptible to ≥1 agent in all but ≤2 categories; and PDR: non-susceptible to all categories of antimicrobial agents. These are currently used for animals in Cambodia. The zone diameter standards suggested by CLSI (2020) were interpreted to classify the isolates as susceptible, intermediate, or resistant.

6. Statistical analysis

The significance of differences was determined using analysis of variance. A *P*-value of <0.05 was considered statistically significant. The least-squares difference test was conducted to assess the significance of variations in the physicochemical properties of the samples. Pearson correlation was used to investigate the relationships between pH, salt content, and a_w in fermented fish and vegetable products. The Statistical Package for Social Sciences, SPSS Version 26.0.0 for Windows, was used for all statistical studies (IBM Co., Somers, NY, USA).

Results

1. pH, salt concentration, and water activity of fermented fish and vegetable samples

The physicochemical characteristic parameters, including pH, a_w, and salt content of fermented samples, were measured as shown in Table 1. The pH values of these four categories of the fermented product differed significantly ($P < 0.05$). The mean pH value of *Chruok* was significantly the lowest (4.44 ± 0.51), followed by *Pha-ork* (4.86 ± 0.48), *Mam* (5.08 ± 0.30), *Prahok* (6.17 ± 0.36), and *Trey Praherm* (6.53 ± 0.69). The pH values of *Pha-ork* and *Mam* exhibited no statistically significant difference ($P > 0.05$). The pH results were consistent with the previous studies, reporting the pH of fermented vegetables ranged 3.6-6.5 (Chrun et al. 2017, Ly et al. 2020). The pH value of fermented fish, ranging 3.9-6.7, agrees with that of other studies: for example, Myanmar *ngaphae-chin* (Tanasupawat & Visessanguan 2014), Philippine *patis*, Thai *Pla-som* (Phithakpol et al. 1995), and Taiwanese fish products (Tsai et al. 2006). The salinity of *Chruok* (2.30 ± 1.13%) and *Trey Praherm* (3.92 ± 4.19%) were statistically comparable ($P > 0.05$), but they significantly differed from *Pha-ork* (8.65 ± 3.27%), *Mam* (8.93 ± 3.70%), and *Prahok*, which had the greatest salt concentration (21.2 ± 5.74%) ($P < 0.05$).

The water activities among the categories of fermented products differed significantly ($P < 0.05$). *Chruok* had the highest water activity (0.92 ± 0.03), followed by *Trey Praherm*, *Pha-ork*, *Mam*, and *Prahok* with values of 0.84 ± 0.04, 0.81 ± 0.04, 0.77 ± 0.04, and 0.74 ± 0.05, respectively. According to Pearson correlation test, the relationship between "pH-a_w," "pH-Salt%," and "a_w-Salt%" in each category of tested fermented samples was weak (r = 0.09-0.58). However, among all samples (n = 220), a moderate correlation was observed between pH values and salt contents (r = 0.64, $P < 0.05$) and between pH and a_w values (r = −0.64, $P < 0.05$).

Table 1. Physicochemical properties among the categories of 220 fermented fish and vegetables

Khmer name (number of sample)	Category of sample	pH		a_w		Concentration of salt (%)	
		Range	Mean	Range	Mean	Range	Mean
Chruok (*n=102*)	Fermented vegetables	2.86 - 6.54	4.44±0.51^a	0.80 - 0.96	0.92±0.03^e	0.52 - 8.18	2.30±1.13^a
Prahok (*n=26*)	Fish paste	5.50 - 6.74	6.17±0.36^c	0.70 - 0.83	0.74±0.05^a	8.90 - 33.13	21.2±5.74^c
Pha-ork (*n=64*)	Fermented fish	3.90 - 6.17	4.86±0.48^b	0.75 - 0.94	0.81±0.04^c	2.58 - 20.48	8.65±3.27^b
Mam (*n=23*)	Fermented fish	4.51 - 5.67	5.08±0.30^b	0.72 - 0.86	0.77±0.04^b	1.90 - 18.71	8.93±3.70^b
Trey Praherm (*n=5*)	Salted fish	5.40 - 7.16	6.53±0.69^d	0.80 - 0.90	0.84±0.04^d	1.76 - 11.41	3.92±4.19^a

The different alphabetic letter in each column means "significant difference" with a *P*-value of <0.05.

A significant negative correlation (r = −0.78, $P < 0.05$) was observed between the salt concentrations and a_w values.

2. Prevalence of contaminated bacteria in fermented fish and vegetables

The prevalence of isolated bacteria in each of the five fermented foods is shown in Table 2. *E. coli* is the most prevalent contamination among the bacteria. *Trey Praherm* (salted fish) had the highest presence of *E. coli* (60%), followed by *Mam*, *Prahok*, *Pha-ork*, and *Chruok* samples at 52%, 35%, 33%, and 28%, respectively. No significant difference in *E. coli* detection rates among five categories of foods was observed ($P > 0.05$). Moreover, the second range of harmful bacteria was *Proteus spp*., which was detected in 40% of *Trey Praherm*, followed by *Mam, Chruok, Prahok,* and *Pha-ork* at 35%, 18%, 15%, and 11%, respectively. Other bacteria discovered in low percentages (2%-4%) were *Providencia spp.* and *Citrobacter spp.*. However, *Citrobacter spp.* is detectable in *Trey Praherm* (20%), and *Salmonella enterica* was found in one of the five *Trey Praherm* samples.

We could isolate 19 kinds of bacteria from 220 collected samples (Table 3). *E. coli* was the most common contamination in the tested fermented products (34%), followed by *Proteus spp., Providencia spp.,* and *Citrobacter spp.* at 18%, 3%, and 3%, respectively. Other bacteria from the Enterobacteriaceae family, including *Raoultella planticola, Raoultella terrigena*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *Ewardsiella tarda*, *Klebsiella oxytoca*, and *Kluyvera cryocrescens*, accounted for approximately 6% of the total samples. *Salmonella enterica* was isolated from one sample. For all of the isolated bacteria, no significant difference ($P > 0.05$) in the rate of positive samples between pH <5 samples and pH ≧5 samples. No significant difference ($P > 0.05$) in the rate of positive samples between the salt concentration of <10% samples and salt concentration ≧10% samples for all isolated bacteria.

3. Salt and pH tolerance

The tolerance of the identified bacteria isolated from fermented vegetables and fish was tested at various salt concentrations (4%, 6%, 8%, and 10%). Table 4 shows that the identified *E. coli*, *Enterobacter cloacae, Citrobacter spp., Klebsiella oxytoca, Proteus spp., Providencia spp., Raoultella planticola, Raoultella terrigena,* and *Salmonella enterica* may persist at 8% maximal salt concentration after incubation. Conversely, *Kluyvera cryocrescens* and *Morganella morganii* can survive at 6% salt concentration. Only *Enterobacter aerogenes*, *Ewardsiella tarda,* and *Kluyvera cryocrescens* can grow at 4% salt concentration.

Table 4 shows that most bacteria, including *E. coli, Enterobacter cloacae, Citrobacter spp., Klebsiella oxytoca, Kluyvera cryocrescens, Morganella morganii, Proteus spp., Providencia spp., Raoultella planticola,* and *Raoultella terrigena*, could not survive at ≤4 pH. However, all isolated strains could grow to 100% at 5 and 6 pH, except *Enterobacter aerogenes, Ewardsiella tarda,* and *Salmonella enterica,* which were absent at 5 pH.

4. Antibiotic resistance of the identified species

The paper disk diffusion method evaluated all identified isolates from fermented vegetables and fish for antibiotic resistance (Table 5). *E. coli* (50%-100%) was the most resistant to 8 of the 17 different control antibiotics. Over 50% of isolated *Proteus spp.* were colistin sulfate-, oxytetracycline-, oxacillin-, sulfamethoxazole-, and tetracycline-resistant, except *P. penneri* and *P. vulgaris*, which were susceptible to tetracycline. The isolated *Salmonella enterica* were penicillin-, colistin sulfate-, and oxacillin-resistant. Table 5 shows that isolates of *E. coli*, *P. mirabilis*, *P. penneri*, and *P. vulgaris* were MDR to β-lactams, cyclic polypeptides, sulfonamides, and tetracyclines.

Table 2. The prevalence of *E. coli* and other Enterobacteriaceae family in fermented fish and vegetables

Bacteria	Categories of fermented fish and vegetables: *Chruok* (102 Samples)		*Prahok* (26 Samples)		*Pha-ork* (64 Samples)		*Mam* (23 Samples)		*Trey Praherm* (5 Samples)		Total (133 Samples)	
	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)
Citrobacter spp.	2	(2)	1	(4)	2	(3)	0	(0)	1	(20)	2	(2)
E. coli	29	(28)	9	(35)	21	(33)	12	(52)	3	(60)	49	(37)
Proteus spp.	18	(18)	4	(15)	7	(11)	8	(35)	2	(40)	21	(16)
Providencia spp.	5	(5)	0	(0)	1	(2)	0	(0)	0	(0)	4	(3)
Other species	4	(4)	5	(19)	1	(2)	2	(9)	1	(20)	6	(5)

The positive numbers and percentages of the four major kinds and the total of the other bacteria for each of the five kinds of foods are shown.

Table 3. Isolated bacteria from 220 fermented fish and vegetable samples

Identified bacteria	Number (percentage) of positive samples (All)		Number (percentage) of positive samples *n = 133* (pH<5)		Number (percentage) of positive samples *n = 87* (pH≧5)		Number (percentage) of positive samples *n = 169* (Salt<10%)		Number (percentage) of positive samples *n = 51* (Salt≧10%)	
Citrobacter amalonaticus	1	(0.5)	1	(1)	0	(0)	1	(1)	0	(0)
Citrobacter youngae	1	(0.5)	0	(0)	1	(1)	0	(0)	1	(2)
Citrobacter sedlakii	1	(0.5)	0	(0)	1	(1)	0	(0)	1	(2)
Citrobacter freundii	3	(1.4)	1	(1)	2	(2)	3	(2)	0	(0)
Escherichia coli	74	(33.6)	49	(37)	25	(29)	58	(34)	16	(31)
Enterobacter aerogenes	1	(0.5)	0	(0)	1	(1)	1	(1)	0	(0)
Enterobacter cloacae	1	(0.5)	1	(1)	0	(0)	1	(1)	0	(0)
Edwardsiella tarda	2	(0.9)	2	(2)	0	(0)	2	(1)	0	(0)
Klebsiella oxytoca	2	(0.9)	0	(0)	2	(2)	2	(1)	0	(0)
Kluyvera cryocrescens	2	(0.9)	2	(2)	0	(0)	2	(1)	0	(0)
Morganella moganii	2	(0.9)	0	(0)	2	(2)	1	(1)	1	(2)
Proteus mirabilis	28	(12.7)	18	(14)	10	(11)	25	(15)	3	(6)
Proteus penneri	10	(4.5)	2	(2)	8	(9)	5	(3)	5	(10)
Proteus vulgaris	1	(0.5)	0	(0)	1	(1)	1	(1)	0	(0)
Providencia heimbachae	2	(0.9)	2	(2)	0	(0)	2	(1)	0	(0)
Providencia stuartii	4	(1.8)	2	(2)	2	(2)	4	(2)	0	(0)
Raoultella planticola	1	(0.5)	1	(1)	0	(0)	1	(1)	0	(0)
Raoultella terrigena	1	(0.5)	0	(0)	1	(1)	0	(0)	1	(0)
Salmonella enterica	1	(0.5)	0	(0)	1	(1)	1	(1)	0	(0)

The positive rate of each of the 19 kinds of bacteria isolated from 220 collected samples is shown.
The samples' rates in each of the categories (<5 and ≧5 pH, and <10% and ≧10% salt concentration) are also shown.

Discussion

1. Physicochemical characteristics of fermented fish and vegetables

The physicochemical results in Table 1 show that fermented vegetables differ from fermented fish. Fermented fish samples, including *Prahok*, *Pha-ork*, *Mam*, and *Trey Praherm*, have a higher pH than that of fermented vegetables (*Chruok*). This is probably because protein-rich foods have a high buffering capacity, making fermented fish more resistant to pH changes compared with fermented vegetables (Mennah-Govela et al. 2019). Additionally, *Trey Praherm,* Myanmar's traditional semi-dried salted fish product, had the highest pH value

Table 4. Salt and pH tolerance of identified bacteria from fermented samples

Bacteria	Number of identified strains	Number (Percentage) of grown strains						
		Salt concentration				pH		
		4%	6%	8%	10%	4	5	6
Citrobacter freundii	3	3 (100)	3 (100)	3 (100)	0 (0)	0 (0)	3 (100)	3 (100)
Citrobacter amalonaticus	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Citrobacter youngae	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Citrobacter sedlakii	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Escherichia coli	74	74 (100)	71 (96)	67 (91)	0 (0)	0 (0)	74 (100)	74 (100)
Enterobacter aerogenes	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Enterobacter cloacae	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Ewardsiella tarda	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
Klebsiella oxytoca	2	2 (100)	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)
Kluyvera cryocrescens	2	2 (100)	1 (50)	0 (0)	0 (0)	0 (0)	2 (100)	2 (100)
Morganella moganii	2	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	2 (100)
Proteus mirabilis	28	28 (100)	28 (100)	25 (89)	0 (0)	0 (0)	28 (100)	28 (100)
Proteus penneri	10	10 (100)	10 (100)	5 (50)	0 (0)	0 (0)	8 (80)	10 (100)
Proteus vulgaris	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
Providencia heimbachae	2	2 (100)	2 (100)	1 (50)	0 (0)	0 (0)	2 (100)	2 (100)
Providencia stuartii	4	4 (100)	4 (100)	3 (75)	0 (0)	0 (0)	4 (100)	4 (100)
Raoultella planticola	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Raoultella terrigena	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Salmonella enterica	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)

Each of the same names of bacteria was isolated from different fermented foods.
The numbers in "( )" show percentages of grown strains in each kind of bacteria.

among fermented fish because of relatively short fermentation (yegyo ngapi); its pH ranges 5.8-6 (Kobayashi et al. 2016). The significant pH increase in fermented fish compared with fermented vegetables (Table 1) was attributed to the higher ammonia nitrogen and urea content by degrading small peptides throughout the fermentation period (Zhao et al. 2019). Conversely, vegetable fermentation produces lactic acid bacteria that ferment higher sugars, leading to pH lowering (Bautista-Gallego et al. 2020, Vatansever et al. 2017).

The average fermented fish salt concentration ranged 3.92 ± 4.19%-21.2 ± 5.74%. In a study of Cambodian fermented food characteristics (Ly et al. 2020), the a_w value (0.76-0.97) and salt content value (2%-13%) of fermented vegetables were similar to this study's results (Table 1). The a_w value of fermented fish

Table 5. Antibiotic resistance profile of isolated bacteria by paper disc diffusion method

Antimicrobial category	Antimicrobial agent	Number (percentage) of resistant strains *E. coli* (74 strains)	*P. mirabilis* (28 strains)	*P. penneri* (10 strains)	*P. vulgaris* (1 strains)	*S. enterica* (1 strains)
Acetamide antibiotic	Florfenicol	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aminoglycosides	Gentamicin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
β-lactams	Amoxycillin	39 (53)	0 (0)	0 (0)	0 (0)	0 (0)
	Ampicillin	38 (51)	0 (0)	0 (0)	0 (0)	0 (0)
	Oxacillin	74 (100)	28 (100)	10 (100)	1 (100)	1 (100)
	Penicillin G	72 (97)	0 (0)	0 (0)	0 (0)	1 (100)
Cephalosporins	Cefoxitin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Ceftazidime	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Cephazolin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Polypeptides cyclic	Colistin Sulphate	46 (62)	28 (100)	10 (100)	1 (100)	1 (100)
Quinolones	Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Enrofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Norfloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sulfonamides	Sulfamethoxazole	37 (50)	14 (50)	5 (50)	1 (100)	0 (0)
Tetracyclines	Oxytetracycline	49 (66)	25 (89)	5 (50)	1 (100)	0 (0)
	Tetracycline	43 (58)	22 (79)	0 (0)	0 (0)	0 (0)

Drug resistance of the strains was determined by comparing the diameter of inhibitation zone and zone diameter breakpoints shown on CLSI (2020).

was comparable with that of Indonesian fermented fish (0.75-0.93) (Petrus et al. 2013). While, the highest a_w value was obtained from fermented vegetables (*Chrouk*), the lowest was obtained from *Prahok* with a mean of 0.92 ± 0.03 and 0.74 ± 0.05, respectively. Contrarily, the lowest and the highest salt content was found in *Chrouk* and *Prahok* with a mean of 2.30 ± 1.13 and 21.2 ± 5.74, respectively (Table 1). Panagou (2006) found that the a_w of Greek dry-salted olives decreased during the salting process. Thus, the high a_w value in *Chrouk* is due to the low salt concentration and vice-versa for *Prahok*.

2. Prevalence of contaminated bacteria in fermented fish and vegetables

E. coli prevalence was found highest (34%) in 220 isolated bacteria from fermented fish and vegetables, followed by *Proteus spp.* (Table 3). This study also shows that the Enterobacteriaceae family is comparable with *Adjuevan*, a traditional Ivorian fermented fish (Koffi-Nevry & Koussémon 2012). Additionally, Table 2 shows the Property of *E. coli* in fermented foods (or fish and vegetables) is well-matched with the study of Ly et al. (2020) on the characteristics of several Cambodian fermented foods. The presence of *E. coli* in these foods indicates that fecal contamination occurred during processing, including using contaminated water (Massa et al. 1988) and poor hygiene practices (Waisundara et al. 2016). Hence, these results showed that hygiene practices are poor in food fermentation in Cambodia. Intrinsic factors, pH, a_w, and salt concentration in fermented foods affect the survival of bacteria. *E. coli* survives a pH ranging 4.3-10 (Bintsis 2017). Enterobacteriaceae can grow in pH ranging 3.8-9.0 and >0.94 a_w (Baylis 2011). In this study, the pH range and a_w value in the collected fermented vegetables was between 2.9 and 6.5 and 0.80 and 0.96, respectively. These conditions favor the survival of *E. coli* and other Enterobacteriaceae. Since fermented foods are typically stored at room temperature without expiration date (Ly et al. 2018), consuming Cambodian fermented vegetables might cause foodborne illness. Also, while fermented large-size fish products are often cooked before consumption, fermented small-sized fish and fermented vegetables are commonly consumed directly. Therefore, fermented small-sized fish and fermented vegetables may cause more foodborne diseases.

We could not determine both the significant difference in the positive rate of bacterial contamination between the samples of <5 and ≧5 pH and the samples of <10% and ≧10% salt concentrations (Table 3). Although low pH and high concentrations of salt inhibit the growth of bacteria, they are insufficient to kill them. The difference in the contamination rates shown in Table 3 may reflect the hygiene conditions and procedures of production.

3. Salt and pH tolerance of the isolated strains

All isolates from fermented vegetables were subjected to different salt concentrations (Table 4). These salt tolerance tests showed that 91% of *E. coli* and most Enterobacteriaceae isolated from fermented samples were tolerant to 8% salt concentration. These results support a hypothesis that *E. coli* strains could grow at salt concentrations of 5%-10%, suggesting that bacteria can develop higher salinity tolerance levels after exposure to saline conditions for several generations (How et al. 2013). *E. coli* and other Enterobacteriaceae in the environment are probably continuously exposed to salinity conditions in fermented foods, resulting to higher persistence and salinity tolerance and to lower inhibiting effect of salt. The salt content range among fermented food categories was also <10%, indicating a possible survival of tolerant *E. coli* and other Enterobacteriaceae, posing higher risk to foodborne illness.

Table 4 shows that 100% of *E. coli* adapted to and grew well at >4-6 pH. Xu et al. (2020) also reported that the acid limit for *E. coli* to grow is 4.0 and 4.5 pH in rich medium and in minimal medium, respectively. Moreover, most Enterobacteriaceae species, except *Enterobacter aerogenes, Ewardsiella tarda,* and *Salmonella enterica*, were intolerant to ≤5 pH. Studies of Abbas et al. (2014) and Yang et al. (2020) stating that Enterobacteriaceae bacteria grow well at a pH ranging >4-7 also agree with these results. The adaptation of *E. coli* and most Enterobacteriaceae to acid allows them to survive under the acidic condition of fermented vegetables and fish, where the pH values are inherently low. Bacteria could resist more acidic conditions when exposed to non-lethal acid pH (Lin et al. 1996). Therefore, *E. coli* and Enterobacteriaceae on fermented vegetables and fish may be exposed to moderate acid conditions, rendering them more tolerant to low pH levels. Presumably, consuming Cambodian fermented vegetables and fish are dangerous due to the survival of acid-tolerant *E. coli* and some Enterobacteriaceae.

4. Antibiotic resistance of the identified species

All isolates were oxacillin-resistant, followed by colistin sulfate, oxytetracycline, and sulfamethoxazole (Table 5). Conversely, all of the tested bacteria were susceptible to cefoxitin, ceftazidime, cephazolin, ciprofloxacin, enrofloxacin, florfenicol, gentamicin, levofloxacin, and norfloxacin. Most isolated *E. coli* strains were resistant to eight antibiotics (Table 5). 58% of *E. coli* were tetracycline-resistant compared with 33%, as highlighted by Sainz et al. (2001). Also, the European Centre for Disease Prevention and Control's (ECDC) AMR surveillance data revealed an increased antibiotic resistance among invasive *E. coli* isolates (Erjavec 2019). Isolated strains of *Proteus spp.* were colistin sulfate-, oxytetracycline-, oxacillin-, sulfame thoxazole-, and tetracycline-resistant, except *P. penneri* and *P. vulgaris*, which are susceptible to tetracycline. This was consistent with the previous study of Farmer et al. (2010).

Table 5 shows that >50% of the isolated *E. coli, P. mirabilis, P. penneri, and P. vulgaris* were resistant to β-lactams, polypeptides cyclic, sulfonamides, and tetracyclines. Large doses of antibiotics used for human therapy, livestock, and fish in aquaculture led to the selection of pathogenic multidrug-resistant bacteria. These bacteria may also arise from increased expression of genes that code for multidrug efflux pumps, which extrude various drugs (Nikaido 2009). *E. coli* with expressing *AcrB* genes can excrete most antibiotic dyes, detergents, and solvents (Poole & Srikumar 2001, Li & Nikaido 2004). The resistance of bacteria to these antimicrobials is probably associated with the misuse of these antibiotics in public health and animal husbandry. These antimicrobial-resistant bacteria in Cambodian fermented foods could arise a public health problem; so, a search for more advanced antibiotics against these resistant bacteria is necessary.

Conclusion

This study investigated on the quality of Cambodian fermented vegetables and fish and the antibiotic resistance of the bacteria isolates, including *E. coli* and other Enterobacteriaceae bacteria. pH variations, salt concentration, and a_w values could be related to the survival of Enterobacteriaceae species. Some isolated and identified strains showed salinity tolerance up to 8% concentration; although, they were all inhibited at 4 pH. The prevalence of *E. coli* and *Proteus spp.* was found to be the highest among the bacterial isolates in fermented products, and more than half of each isolate exhibited resistance to the tested antibiotics, possibly including MDR. The research results suggest that fermented vegetables and fish are likely sources of and a vehicle for the transmission of antibiotic-resistant bacteria, leading to foodborne illnesses. Also, basic sanitary measures should be applied during the processing line to prevent the potential incidents of consuming contaminated fermented products. Further research should focus on the techniques that could make fermented foods in Cambodia safer to consume.

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References

Abbas, S. Z. et al. (2014) Isolation and characterization of arsenic-resistant bacteria from wastewater. *Braz. J. Microbiol.*, **45**, 1309-1315.

Al-Dhabaan, F. A. M. & Bakhali, A. H. (2017) Analysis of the bacterial strains using Biolog plates in the contaminated soil from the Riyadh community. *Saudi J. Biol. Sci.*, **24**, 901-906.

Ananchaipattana, A. et al. (2012) Prevalence of foodborne pathogens in retailed foods in Thailand. *Foodborne Pathog. Dis.*, **9**, 835-840.

Ananchaipattana, A. et al. (2012) Serotyping, RAPD grouping, and antibiotic susceptibility testing of S*almonella enterica* isolated from retail foods in Thailand. *Food Sci. Technol. Res.*, **20**, 905-913.

Bautista-Gallego, J. et al. (2020) Role of lactic acid bacteria in fermented vegetables. *Grasas y Aceites*, **71**, 1-9.

Baylis C. et al. (2011) The Enterobacteriaceae and their significance to the food industry. *ILSI Europ. Rep. Series*, **2011**, 1-48.

Bintsis, T. (2017) Foodborne pathogens. *AIMS Microbiol.*, **3**, 529-563.

Chrun, R. et al. (2017) Microbiological hazard contamination in fermented vegetables sold in local markets in Cambodia. *Biocontrol Sci.*, **22**, 181-185.

Chuon, M. R. et al. (2014) Microbial and chemical properties of Cambodian traditional fermented fish products. *J. Sci. Food Agric.*, **94**, 1124-1131.

Clinical and Laboratory Standards Institute (2020) CLSI M100-ED29: 2021 Performance standards for antimicrobial susceptibility testing, 30th ed. Waune, PA, USA.

Dimidi, E. et al. (2019) Fermented foods: Definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. *Nutrients*, **11**, 1806.

Doyle, M. E. (2015) Multidrug-resistant pathogens in the food supply. *Foodborne Pathog. Dis.*, **12**, 261-279.

Erjavec, M. S. (2019) Introductory chapter: The versatile Escherichia coli. *The Universe of Escherichia coli*. Intech Open Ltd. London, UK.

FAO-WHO. (2004) Practical actions to promote food safety. Regional Conference on Food Safety for Asia and the Pacific., 24-27 May 2004.

Farmer, J. J. et al. (2010) The Enterobacteriaceae: General characteristics. *Topley & Wilson's Microbiol. & Microbial Infect*.

Foster, J. (2004) *Escherichia coli* acid resistance: Tales of an amateur acidophile. *Nat. Rev. Microbiol.*, **2**, 898-907.

Hoorfar, J. & Baggesen, D. (1998) Importance of pre-enrichment media for isolation of *Salmonella* spp. from swine and poultry. *FEMS Microbiol. Lett.*, **169**, 125-130.

How, J. A. et al. (2013) Adaptation of *Escherichia coli* ATCC 8739 to 11% NaCl. *Dataset Pap. Biol.*, 1-7.

Isola, L. A. et al. (2022) A review on fermented aquatic food storage quality based on heat treatment and water retention technology. *Food Sci. Technol.* (Brazil), **42**, 1-12.

Kobayashi, T. et al. (2016) Diversity of the bacterial community in Myanmar traditional salted fish yegyo ngapi. *World J. Microbiol. Biotechnol.*, **32**, 4-5.

Koffi-Nevry, R. & Koussémon, M. (2012) Microbiological composition, processing and consumer's characteristics of adjuevan, a traditional ivorian fermented fish. *Tropocultura*, **30**, 9-14.

LeGrand, K. et al. (2020) Tradition and fermentation science of prohok, Cambodia's ethnic fermented fish product. *J. Ethn. Foods*, **7**, 1-19.

Li, X. Z. & Nikaido, H. (2004) Efflux-mediated drug resistance in bacteria. *Drugs*, **64**, 159-204.

Lin, J. (1996) Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl. Environ. Microbiol.*, **62**, 3094-3100.

Ly, D. et al. (2018) Significance of traditional fermented foods in the lower Mekong subregion: A focus on lactic acid bacteria. *Food Biosci.*, **26**, 113-25.

Ly, D. et al. (2020) Biogenic amine contents and microbial characteristics of Cambodian fermented foods. *Foods*, **9**, 198-217.

Magiorakos, A. P. et al. (2012) Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinic. Microbiol. Infect*., **18**, 268-281.

Manyi-Loh, C. et al. (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules*, **3**, 795.

Massa, S. et al. (1988) Isolation of *Yersinia enterocolitica* and related species from river water. *Zentralbl. Mikrobiol*., **14**, 3575-3581.

Mennah-Govela, Y. A. et al. (2019) Buffering capacity of protein-based model food systems in the context of gastric digestion. *Food Funct*., **10**, 6074-6087.

Mores Rall, V. L. et al. (2005) Evaluation of three enrichment broths and five plating media for Salmonella detection in poultry. *Braz. J. Microbiol*., **36**, 147-150.

Nikaido, H. (2009) Multidrug resistance in bacteria, *Ann. Rev. Biochem*., **78**, 119-146.

Panagou, E. Z. (2006) Greek dry-salted olives: Monitoring the dry-salting process and subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4°C and 20°C. *LWT Food Sci. Technol*., **39**, 323-330.

Peristiwati, S. Y. & Trinaya, C. (2019) Isolation and identification of potential culturable probiotics bacteria from intestine of Anguilla bicolor. *J. Phys. Conf. Ser*., **1280**, 2.

Petrus, et al. (2013) Physicochemical characteristics, sensory acceptability, and microbial quality of Wadi betok a traditional fermented fish from South Kalimantan, Indonesia. *Int. Food Res. J*., **20**, 933-939.

Phithakpol, B. et al. (eds.) (1995) The Traditional fermented foods of Thailand, Institute of Food Research and Product Development, Kasetsart University, Bangkok.

Poole, K. & Srikumar, R. (2001) Multidrug efflux in *Pseudomonas aeruginosa*: Components, mechanisms, and clinical significance. *Curr. Top. Med. Chem*., **1**, 59-71.

Pusporini, A. R. & Pasuwan, P. (2020) Microbiological evaluation of Thai fermented fish (Pla-ra) production contact surfaces. *Asia-Pac. J. Sci. Technol*., **25**, 1-9.

Reed, T. A. N. et al. (2019) Antimicrobial resistance in Cambodia: A review. *Int. J. Infect. Dis*., **85**, 98-107.

Sainz, T. et al. (2001) Survival and characterization of *Escherichia coli* strains in a typical Mexican acid-fermented food. *Int. J. Food Microbiol*., **71**, 169-176.

Sezey, M. & Adun, P. (2019) Validation of mohr's titration method to determine salt in olive and olive brine. *J. Turk. Chem. Soc. A: Chem*., **6**, 329-334.

Sichewo, P. R. et al. (2014) Isolation and identification of pathogenic bacteria in edible fish : A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. *Int. J. Curr. Microbiol*., **3**, 897-904.

Soeung, R. et al. (2017) Detection of coliforms, *Enterococcus* spp. and *Staphylococcus* spp. in fermented vegetables in major markets in Cambodia. *Acta Hortic*., **1179**, 139-142.

Tanasupawat, S. & Visessanguan, W. (2014) Fish fermentation. *Seafood Processing: Technol*., *Quality*, *and Safety*, **24**, 177-207.

Thompson, L. et al. (2021) Towards improving food safety in Cambodia: Current status and emerging opportunities. *Glob. Food Secur*., **31**, 100572.

Tsai, Y. H. et al. (2006) Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food Chem*., **98**, 64-70.

Vatansever, S. et al. (2017) The effect of fermentation on the physicochemical characteristics of dry-salted vegetables. *J. Food Res*., **6**, 32.

Vijayakumar, P. P. & Adedeji, A. (2017) Measuring the pH of food products. *Univ. Kentucky*, *College Agric*., *Food Environ*., **7**, 1-2.

Xu, Y. et al. (2020) An acid-tolerance response system protecting exponentially growing *Escherichia coli*. *Nat. Commun*., **11**, 1-13.

Yang, Q. et al. (2020) Effects of amino acid decarboxylase genes and pH on the amine formation of enteric bacteria from Chinese traditional fermented fish (Suan Yu). *Front. Microbiol*., **11**, 1130.

Zekar, F. M. et al. (2017) From farms to markets: Gram-negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of North Africa. *Front. Microbiol*., **8**, 1569.

Zhao, C. C. et al. (2019) Changes in protein compositions and textural properties of the muscle of skate fermented at 10°C. *Int. J. Food Prop.*, **22**, 172-184.