Original Article

MICROBIAL EVALUATION AND ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC BACTERIA ISOLATED FROM "PLA-SOM" AND "PLA-CHOM", THAI RICE-FERMENTED FISH PRODUCTS

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Abstract

Pla-som and pla-chom are Thai traditional fermented fish, which is produced from fish, sugar, salt and roasted rice and is fermented with natural lactic acid bacteria, therefore pathogenic bacteria isolation from these traditional products are sparely reported. This study was aimed to evaluate contamination of pathogenic bacteria and their antibiotic susceptibility from pla-som (n = 15) and pla-chom (n = 15). According from colony, Gram □s staining and biochemical characteristics, pathogenic bacteria were isolated and identified; tested for antibiotic susceptibility. Pathogenic bacteria isolate from pla-som (n = 32) were higher rather than pla-chom (n = 12). Suspected pathogenic bacteria were Staphylococcus aureus and Escherichia coli isolated from both of rice-fermented fishes while Salmonella typhimurium, Listeria monocytogenes and K. pneumoniae were commonly contaminated in pla-som. Salt-tolerate pathogens including Vibrio sp. and Aeromonas hydrophila were also identified. Fifty and twenty of pathogenic bacteria were isolated from pla-som and pla-chom, which were determined on susceptibility with fifteen antibiotic discs. Some of pathogenic bacteria were resisted to three or more antibiotics and defined as multidrug resistance (MDR) bacteria. Various pathogenic bacteria including S. aureus, E. coli, K. pneumoniae, S. typhimurium, A. hydrophila, Vibrio sp. and Proteus sp., were antibiotic resistance. Vibrio sp. and A. hydrophila were MDR bacteria with five or more antibiotic resistances. The finding is notified on awareness of recipe in consumers and regarding on food safety of traditional fermentation process in local manufacturers.

Keywords: pla-som, multidrug resistance (MDR), pathogenic bacteria, Thai fermented fish.

INTRODUCTION

Fermentation is common food preserving method, which is inhibit of spoilage microbial growth by organic acids and by-product metabolites. As a result, these products generally have a longer shelf life than their original food [1, 2]. Fermented food is providing health promotion including lowering of blood cholesterol, immunomodulation, etc. However, food-borne illness from fermented food consumption had reported, which is due to contamination of bacteria and its toxins [3].

Particularly, food-borne illness is occurred in food products from local household fermentation [4].Pla-som is Thai traditional fermented fish, which is produced from fish, sugar, salt and roasted rice (sometime contained with spices) and is fermented with natural microbial flora.

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Ingredient and process of pla-chom is similar to pla-som, however there is use tiny fishes as raw material, while pla-som is processed from large flesh or whole of fish. Amount of lactic acid bacteria are preserving pla-som, which is depended on salt concentration [5]. Recently, of traditional Thai fermented foods are lack of commercialize starter culture of lactic acid bacteria and majority of fermented foods are produced from small scale production [6]. According to the Thai Agricultural Standard, Thai fermented fish should meet in criteria of requirements including physical characteristics, salt content, food additives, and contaminants i.e., toxic metals and microbials. Traditional fermentation processes i.e., raw ingredient preparation, personnel hygiene and practices, food packaging which is risk to contaminate with various bacteria including pathogenic bacteria [3, 7]. Pathogenic bacteria including Bacillus cereus, Clostridium perfringens, Escherichia coli, Salmonella spp., and Staphylococcus aureus are restricted bacterial contaminants according to the Thai Agricultural Standard [8, 9]. Isolation of pathogenic bacteria from pla-som and pla-chom, Thai rice-fermented fish products are sparely reported when compare with pla-ra, Thai fermented fish without rice contained [8, 9]. The antibiotic resistance of isolated bacteria is also interesting to determine in this study. Because of their can affect public health due to transmission or cross-contamination of foodborne diseases, which are depended on fermentation process, personnel hygiene and device-related contaminants [10, 11]. Hence, we were concerning on bacterial contamination isolated from this Thai rice-fermented fish products. Isolated pathogenic bacteria were further determined antibiotic susceptibility. The finding was provided information and the guideline for local food manufacturers for controlling of fermentation process, which is meet to food quality. In addition, the finding will report and notify on consumers and public health policy on controlling of foodborne diseases.

MATERIALS AND METHODS

Sample Collection and Preparation

Pla-som (n = 15) and pla-chom (n = 15) were purchased from six markets located in Bangkok, Thailand. Each fermented fish was weighed (25 g), suspended in 225 ml of 0.1% peptone water (Merck, Darmstadt, Germany) for enrichment (sample-to-broth ratio, 1:10) and inoculated in selective media (Himedia, India) including McConkey agar, xylose lysine deoxycholate (XLD) agar and eosin-methylene blue (EMB) agar at 37 □C for 24 hrs. Isolated colony was sub-cultured in tryptic soy agar (TSA) agar and incubated similar to selective media for biochemical and antibiotic susceptibility tests.

Bacterial Colony Isolation and Biochemical Identification

Bacterial colony isolate was characterized according by size, shape, convex, edge, color, and morphology. Each isolated colony was stained and identified by Gram □s staining and biochemical tests, respectively. Biochemical tests were included catalase, oxidase, string test, citrate, methyl red, Voges-Proskuer test, oxidation-fermentation, lysine, indole, motility, bile esculin, triple sugar iron (TSI), which were identified pathogenic Gram □s positive bacteria, Gram □s negative Enterobacteria and non-fermentative bacilli (NFB). Thiosulfate citrate bile salt sucrose agar (TCBS) and 0%, 3%, 6%, 8% and 10% NaCl in nutrient broth were used to identified Vibrio spp.

Colony, Gram □s staining and biochemical characteristics of bacterial isolates were interpreted and identified according by Bergey□s Manual of Systematic Bacteriology and Clinical and Laboratory Standards Institute (CLSI) [12, 13].

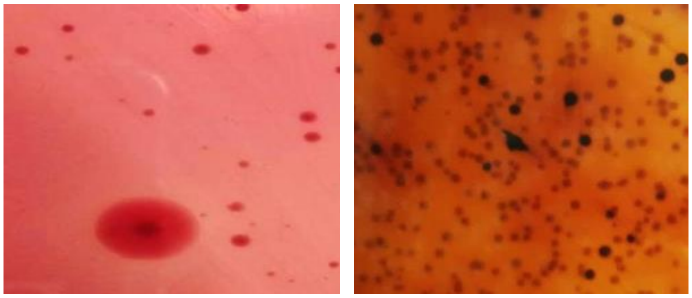
Antibiotic Susceptibility Test

Pathogenic bacteria were evaluated on antimicrobial susceptibility of by agar disc diffusion (Kirby-Bauer) method. Each isolated bacterial colony was sub-cultured in TSA agar, and was inoculated into 4 mL of sterile nutrient broth. Bacterial suspension was adjusted its density with 0.5 McFarland as standard. Then, bacterial suspension was spread over entirely surface of Muller Hinton (MHA) plate, which was dried by standing at room temperature. Fifteen of antibiotic discs (Difco, USA) used in susceptibility test were included amoxicillin/clavulanic acid (30 □g, 20/10 □g), ampicillin (10 □g), amikacin (30 □g), cefoxitin (30 □g), ceftazidime (30 □g), ceftriaxone (30 □g), cefuroxime (30 □g), chloramphenicol (30 □g), ciprofloxacin (5 □g), gentamicin (10 □g), imipenem (10 µg), meropenem (10 µg), piperacillin (30 □g) tetracycline (30 □g), trimethoprim/sulfamethoxazole (25 □g). Inhibition zone was clearing zone surrounded antibiotic disc and measured as diameter (mm). Each inhibition zone value was compared with CLSI reference and interpretated as susceptible (S), intermediate (I) and resistance (R) bacteria against tested antibiotic disc. For this study, intermediate antibiotic susceptibility bacteria were considered as antibiotic resistance and multi-drug resistance (MDR) bacteria were defined as isolated bacteria is resisted three or more different antibiotic discs [14].

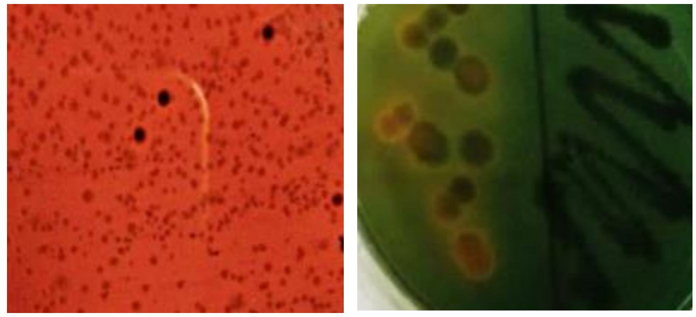
RESULTS AND DISCUSSION

Bacterial Identification and Distribution

Examples of bacterial colonies in each selective media were represented in Fig. 1. Selective media were isolated mainly members of Enterobacteriaceae according by sugar fermentation pattern, color of pH indicators contained in each media, and also pigment formation. According from colony, Gram □s staining and biochemical characteristics, pathogenic bacteria were isolated and identified. Number of pathogenic bacteria isolates from pla-som (n = 32) were higher rather than pla-chom (n = 12), which were unrelated to markets and type of pathogenic bacteria. Suspected pathogenic bacteria were S. aureus and E. coli isolated from both of rice-fermented fishes while S. typhimurium, L. monocytogenes and K. pneumoniae were commonly contaminated in pla-som. We were also found some of Vibrio sp. and Aeromonas hydrophila isolates from pla-som and pla-chom (Table 1).



(a) (b)



(c) (d)

Fig. 1: Bacterial colonies were represented in selective media: (a) McConkey agar, (b) XLD agar, (c) EMB agar (d) TCBS agar

Table 1: Distribution of pathogenic bacteria isolates from Thai rice-fermented fish

Identification/ Fermented fish	Pla-som (n = 15)			Pla-chom (n = 15)		
	M1	M2	M3	M4	M5	M6
Staphylococcus aureus	4	1	2	-	-	1
Bacillus cereus	-	-	1	-	1	-
Listeria monocytogenes	1	2	-	-	-	-
Escherichia coli	3	5	3	-	2	-
Salmonella typhimurium	2	-	1	-	1	-
Klebsiella pneumoniae	-	1	3	1	-	-
Proteus mirabilis	-	-	1	-	2	-
Proteus vulgaris	2	-	1	-	1	-
Enterobacter aerogenes	1	-	-	-	-	-
Vibrio cholerae	-	-	-	1	-	2
Vibrio parahaemolyticus	1	-	-	-	-	1
Vibrio mimicus	1	-	-	-	-	-
Vibrio vulnificus	1	-	-	-	2	-
Vibrio harveyi	2	-	-	-	-	1
Aeromonas hydrophila	1	1	-	1	-	-
Pseudomonas sp.	1	-	-	-	-	-
Total for each market (M)	20	10	12	3	5	4
Total for fermented fish	32			12		
Lactobacillus sp.	3	5	1	-	3	-

Hence, this salt-tolerate pathogens can grow, whereas this product was made from freshwater fish and its salinity was came from variety of salt ingredient. Lactobacillus sp. was contained only in some of products. There was implied that organic acid and preservative metabolites in this product were produced from Lactobacillus sp. and other lactic acid bacteria (LAB). Pla-som and sour fish products in South-East Asia region are contain LAB including Lactobacillus, Lactococcus, Pediococcus, Streptococcus, Enterococcus and Weissella sp. [15-17]. There can inhibit foodborne pathogens and food spoilage microorganisms in pla-som by bacteriocin production i.e., S. salivarius and E. faecalis can inhibit E. coli, S. aureus and Salmonella sp. [15]; W. cibaria 110 can inhibit some of Gram-positive bacteria by Weissellicin 110 (bacteriocin) production [18]. Pathogenic Vibrio sp., S. typhimurium and L. monocytogenes were contaminated in pla-som that may out of standard according by Food and Agriculture Organization (FAO). S. aureus and E. coli were also contaminated in some of rice-fermented fish products, which were concerned on food safety. Therefore, there was lack of quantitative data, which was unable to determine food safety of S. aureus and E. coli contamination in this study [8, 9, 19]. Contamination of Vibrio and Salmonella sp. had also reported in other Thai fermented fish contained garlic or "som-fak" and inhibition of bacterial contamination is depended on amount of lactic acid, salt and garlic [20]. Food quality is not concerning only pathogens and its toxins that cause illness, there is also consider on preferable shelf-life [20]. In case of this study, Thai rice-fermented fish was still desirable characteristics such as flavor, texture and shelf-life. Thus, pathogenic contamination during and after fish fermentation process is one of factor that need to concern [19, 20, 21]. Moreover, acceptability of local fermented food is also important in different cuisine culture. In Thailand and South East Asian countries, fermented food products are valuable by some culture, therefore there are looked disgusting by others. Cuisine culture in North-East of Thailand and nearby area is mostly consumed fermented fish as dietary protein source, however this preserved food has strong odor and flavor as spoilage-like [22, 23].

Antibiotic Susceptibility of Pathogenic Bacteria Isolates

Example of susceptibility test in pathogenic bacterial isolates was shown in Fig. 2. Fifty and twenty of pathogenic bacteria were isolated from pla-som and pla-chom, which were determined on susceptibility with fifteen antibiotic discs. Some of pathogenic bacteria were resisted to three or more antibiotics and defined as multidrug resistance (MDR) bacteria. MDR bacteria isolates from pla-som were E. coli (n = 5, 45%), S. aureus (n = 4, 57%), S. typhimurium (n = 2, 67%), P. vulgaris (n = 2, 67%), V. mimicus (n = 1, 100%), V. vulnificus (n = 1, 100%), V. harveyi (n = 1, 50%), A. hydrophila (n = 1, 50%) and K. pneumoniae (n = 1, 33%). MDR bacteria isolates from pla-chom were V. vulnificus (n = 2, 100%), A. hydrophila (n = 1, 100%), S. typhimurium (n = 1, 100%), P. mirabilis (n = 1, 50%) and E. coli (n = 1, 50%). Some of isolates including V. mimicus, V. vulnificus and K. pneumoniae isolated from pla-som, and V. vulnificus, A. hydrophila and E. coli were rarely occurred MDR bacteria, however there were against resisted five or more antibiotics (Table 2).

In Thailand, pathogenic bacteria contamination in food is common causes of gastro-intestinal diseases. Antibiotic resistance in foodborne microorganisms is worldwide public health concern [24]. Overuse and misuse of antibiotics in livestock farming are remain in environments and there is transfer to water resources and aquaculture [25]. Various microorganisms in fermented foods may act as carriers of antibiotic resistance genes, which are transferred through in food chain between environments, food and human.

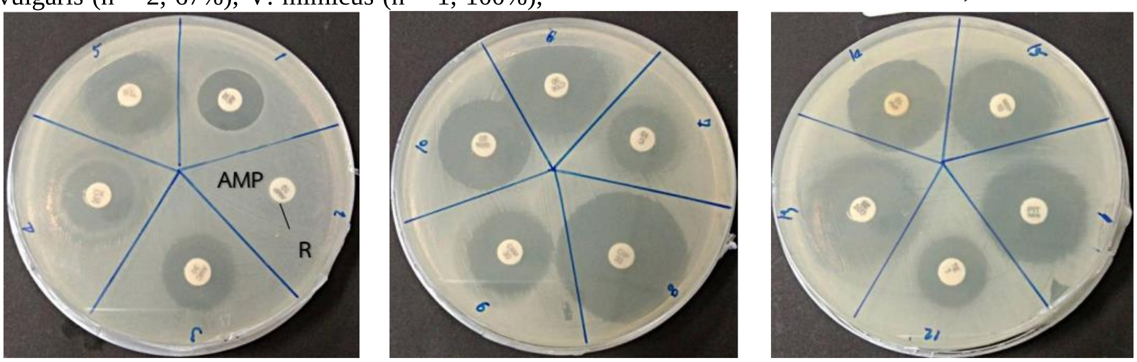


Fig. 2: Bacterial culture was susceptible to fourteen of antibiotic discs and represented as inhibition (clear) zone (mm). Inhibition zone surrounded ampicillin (AMP) disc was narrower than standard range and interpreted as ampicillin resistance (R).

Table 2: Antibiotic resistance patterns of pathogenic bacteria isolated from pla-som and pla-chom

Sample/ Isolates	Antibiotic resistance pattern*	Resistance (%)	MDR (%)
Pla-som			
E. coli (n = 11)	AMP-TE-COT; CX-C-TE; AMP-C-TE; AMP-C-TE; AMP-CXM; AMP-CX; AMP-C; C-CXM; AMP	10 (91%)	5 (45%)
S. aureus (n = 7)	AMP-CX-C-CXM-TE; AMP-CX-TE; AMP-PI-TE; AMP-PI-TE; CIP-TE; AMP; AMP	7 (100%)	4 (57%)
K. pneumoniae (n = 4)	AK-AMP-C-GEN-PI; AMP-CXM; AMP-CX	3 (75%)	1 (33%)
S. typhimurium (n = 3)	AMP-CXM-TE; C-TE-COT; TE	3 (100%)	2 (67%)
P. vulgaris (n = 3)	AMP-CX-CXM-TE-IPM; C-CXM-TE; AMP	3 (100%)	2 (67%)
L. monocytogenes (n = 3)	GEN	1 (33%)	-
A. hydrophila (n = 2)	AMP-TE-IPM; AMP-TE	2 (100%)	1 (50%)
V. harveyi (n = 2)	AMP-CX-TE	1 (50%)	1 (50%)
V. mimicus (n = 1)	AMP-CX-C-CXM-TE	1 (100%)	1 (100%)
V. vulnificus (n = 1)	AMP-CTR-C-CXM-TE	1 (100%)	1 (100%)
E. aerogenes (n = 1)	AMP-C	1 (100%)	-
Pseudomonas sp. (n = 1)	AMP	1 (100%)	-
Pla-chom			
V. cholerae (n = 3)	AMP	1 (33%)	-
V. vulnificus (n = 2)	AMP-AMC-C-CXM-TE-COT-IMP; AMP-C-CXM-COT-IMP	2 (100%)	2 (100%)

P. mirabiris (n = 2)	AMP-TE-COT; AMP-TE	1 (100%)	1 (50%)
E. coli (n = 2)	AK-AMP-CX-CAZ-CTR-CXM-TE; TE	1 (100%)	1 (50%)
S. typhimurium (n = 1)	AMP-C-TE	1 (100%)	1 (100%)
V. parahaemolyticus (n = 1)	AMP	1 (100%)	-
V. harveyi (n = 1)	CX	1 (100%)	-
A. hydrophila (n = 1)	AK-AMP-CAZ-PI-TE-COT	1 (100%)	1 (100%)
S. aureus (n = 1)	AMP	1 (100%)	-
K. pneumoniae (n = 1)	CX	1 (100%)	-

*Pattern of antibiotic drug resistance for each isolate and MDR was defined as bacterial resistant against three or more antibiotics

AK = amikacin; AMP = ampicillin; AMC = amoxiclav; CX = cefoxitin; CAZ = ceftazidime; CTR = ceftriaxone; C = chloramphenicol; CIP = ciprofloxacin; CXM = cefuroxime; GEN = gentamicin; PI = piperacillin; TE = tetracycline; COT = co-trimoxazole; IPM = imipenem; MR = meropenem; MDR = multidrug – resistant bacteria

As our result, bacterial isolated from Thai fermented fish especially from pla-som were MDR characteristics. Thus, antibiotic resistance of pathogenic bacteria may become concerning in food safety of Thai fermented fish particularly from traditional process. In addition, lactic acid bacteria in Thai fermented fish products are also considered on genetic transferring of antibiotic resistances [26, 27, 28]. Further study is need to conduct on fermentation parameters of Thai fermented fish i.e., pH, salinity and lactic acid bacteria contained. Beta-lactamase genes and other common antibiotic resistance genes of MDR bacteria in Thai fermented fish products are also need to determined.

Conclusion

Pathogenic bacteria and their antibiotics resistance were occurred in Thai rice-fermented fish, namely pla-som and pla-chom, which may risk of foodborne illness. Simultaneous antibiotic resistances of various pathogenic bacteria including S. aureus, E. coli, K. pneumoniae, S. typhimurium, A. hydrophila, Vibrio sp. and Proteus sp., were represented in this study. Vibrio sp. and A. hydrophila were rarely isolated MDR bacteria, therefore there were resisted against five or more antibiotics. We were concluded that these fermented fish products may a reservoir of antimicrobial resistance/virulence factors and can transfer through food chain by genetical transmission. The finding is notified on awareness of recipe in consumers and regarding on food safety of traditional fermentation process in local manufacturers.

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