

BACTERIAL CONTAMINATION AND ANTIBIOTIC RESISTANCE IN SUSHI FROM “READY-TO-EAT” JAPANESE RESTAURANTS: PRELIMINARY STUDY IN BANGKOK, THAILAND

Kanittada Thongkao¹, Yuttana Sudjaroen², Penpan Payattikul³

^{1,2}Department of Applied Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok- 10300, Thailand

³Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Abstract

This research was aimed to identify pathogenic bacteria and determine their antibiotic susceptibility from sushi samples were purchased from “ready-to-eat” Japanese restaurants in three zones of Bangkok. Traditional sushi (n = 48), such as salmon, mackerel, shrimp and omelet egg were included in this study. Each sushi piece (25 g) was homogenized and diluted sterile peptone water (1:10). Ten-fold serial dilution enrichments were prepared 10^{-3} - 10^{-7} and the broth culture was plated onto TSA agar to determine aerobic bacterial plate count (APC). Enterobacteriaceae bacteria were identified according by FDA-BAM method. Colony counts of *Staphylococcus* sp. were done according to EN/ISO 6888. *Vibrio* sp. were identified according by Clinical and Laboratory Standards Institute (CLSI) guideline. *Aeromonas* sp. and other non-fermented Gram-negative bacilli were isolated and identified according by Bergey’s Manual of Systematic Bacteriology and CLSI guideline. Antibiotic susceptibility test of bacterial isolates was determined by the Kirby Bauer method; fifteen of antibiotic discs were used and inhibition zones were measured. APC from raw materials topping on sushi rice were greater than 10^7 CFU/g in mackerel and shrimp while the salmon and egg were lower. *Pseudomonas aeruginosa* and *V. mimicus* were multi-drug resistance (MDR) bacteria and can cause human diseases. We were concluded that raw topping sushi, such as raw fish and shrimp were risk of MDR bacteria contamination. MDR genotyping of pathogenic bacteria isolated from raw fish and seafood topping sushi should be conduct for public health control on cleanliness food process and food hygiene.

Keywords: antibiotic resistance, fishery product, multi-drug resistance bacteria, sushi.

INTRODUCTION

Sushi is most popular Japanese foods in Thai consumers. Majority of sushi and sashimi ingredients are come from aquatic products. The fresh raw material is topping on vinegared rice and eaten with soy sauce and wasabi, which had reported antibacterial and anti-fungal properties [1, 2]. Most favorite of sushi is finger-sized rice balls, which is top placing with the raw fish fillet, other seafood products, meat, omelet egg, seaweed and vegetables or other food ingredients. Fish and marine products are containing many valuable nutrients, such as omega 3 as well as good quality protein and vitamins [3, 4]. Consuming of sushi is trend to concerning of food safety, which is more consideration in food cleanliness when use raw or un-cooked through heat.

Raw fish and rice ball are main sushi ingredients, which are consider on risk of pathogenic contamination and there can grow up from food handler [5]. The poor practices of personal hygiene can result in a foodborne illness especially diarrheal disease.

Address for correspondence: Kanittada Thongkao,
Faculty of Science and Technology, Suan Sunandha Rajabhat University,
Bangkok- 10300, Thailand

E-mail address: kanittada.th@ssru.ac.th

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Raw Thai food products can contaminate with pathogenic bacteria including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella* sp. [6, 7] and several reports have identified the risks associated with consuming contaminated food [8, 9]. Therefore, good hygiene on cooking practice and environment are important to ensure the safety of sushi sold to consumers. However, sushi restaurants or sellers are popular in urban area and flavory represent as street stalls or flea markets where are selling as “ready-to-eat” sushi [10, 11]. Hence, we were concerning on cleanliness quality of sushi, which was aimed to isolate pathogenic bacteria from most favored sushi from ready-to-eat Japanese restaurants. Bacterial contamination was identified from most favorite topping, such as salmon, mackerel, shrimp and sweet omelet egg. Isolated bacteria were determined antibiotic susceptibility and further identified antibiotic genes. The finding was provided information and the guideline for cookers, food sellers and food distributors for controlling of food process and quality according by risk assessment of food production for sale distribution which is implement and notify from the Ministry of Public Health.

MATERIAL AND METHODS

Sample Preparation and Bacterial Isolation from Sushi

Sushi samples (n = 48) were collected from “ready to eat” sushi shops or restaurants in three areas of Bangkok (Zone A, B and C), Thailand. The nigiri-sushi combination of seasoned rice with toppings of salmon, mackerel, shrimp and omelet egg (12 pieces for each topping). Each sushi piece was homogenized in liquid form. Each sample was aseptically weighed (25 g) and transferred to a sterile bag. 1% sterile peptone water (Merck, Darmstadt, Germany) was added for enrichment (sample-to-broth ratio, 1:10). Mix well by swirling and brief hand-massage after that incubate 3 hours at 37°C. A ten-fold serial dilution enrichments were prepared at 10⁻³-10⁻⁷ colonies forming unit per ml (CFU/ml). Then, this broth culture was poured onto TSA agar to perform aerobic bacterial plate count (APC) according to ISO 4833. Enterobacteriaceae bacteria were growing well on xylose lysine deoxycholate (XLD), eosin methylene blue (EMD) and McConkey agars (Himedia, India) when incubated at 37°C for 24-48 h. *Salmonella* colonies were counted on three agars after incubation, which were characterized by typical black (H₂S-produced) colonies according by FDA-BAM method [12]. *Staphylococcus* colony were counted on Baird-Parker agar after incubation for 48 to 72 h at 37°C and characterized by EN/ISO 6888. *Listeria* was enriched in half and full Fraser broth (Merck, USA) for 24 h at 30°C and 48 h at 37°C, respectively. Colony identification and count were performed according by EN/ISO 11290. *Bacillus cereus* was cultured and counts in MYP plate; isolated colony was enriched in BHI contained with 1% glucose for enterotoxin study [13]. *Vibrio*

colonies were cultures on thiosulfate citrate bile salt sucrose agar (Himedia, India); and identified by Gram 's staining, oxidase, catalase, lysine indole motility, ornithine decarboxylase, methyl red-vogetprokauer, mannitol, glucose utilization, growth at different NaCl concentration according by Clinical and Laboratory Standards Institute (CLSI) guideline. *Aeromonas* colonies were separated from *Vibrio* spp. by using string test, 0% NaCl growth, 6.5% NaCl Growth, bile esculin test. Other Gram-negative coccobacilli bacteria were cultured on McConkey and XLD agars at 37°C, 24 h; and biochemical identification were explained by Bergey's Manual of Systematic Bacteriology [14]. *Candida* colonies or yeast were cultured on potato dextrose agar (PDA) agar. Biochemical tests were interpreted for bacterial (yeast) identification according by Clinical and Laboratory Standards Institute (CLSI) guideline [15, 16]. Typical colony counts on selective media were also recorded and represented as CFU/ml of enrichment broth.

Antibiotic Susceptibility Test of Isolated Bacteria

Isolated bacteria were determined on susceptible of antibiotics by agar disc diffusion (Kirby Bauer) method. The pure colonies culture of all bacteria from a trypticase soy agar (TSA) were selected into 4 mL sterile nutrient broth normal saline. The bacteria suspension was adjusted the turbidity and density equivalent to 0.5 McFarland standard. The sterile cotton swab was dipped into suspension and spread over the entire surface of Muller Hinton (MHA) plate. Inoculated plates were dried by standing at room temperature. Fifteen of antibiotic discs in susceptibility test were included amoxicillin/clavulanic acid (30 µg, 20/10 µg), ampicillin (10 µg), amikacin (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), piperacillin (30 µg) tetracycline (30 µg), trimethoprim/sulfamethoxazole (25 µg). Inhibition zone was clearing zone surrounded antibiotic disc and measured as diameter (mm). Each inhibition zone value was referenced with CLSI guideline and interpreted 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 two or more different antibiotic discs [15, 16].

RESULT AND DISCUSSION

Microbiological quality of the ready to eat sushi (n = 48) were shown in Table 1. Microbiological quality guide for ready-to-eat foods is acceptable at APC <10⁷ CFU/g standard plate count. The comparison of the raw material topping on rice sushi be found that the mean aerobic bacterial count was greater than 10⁷ CFU/g in mackerel and shrimp while the salmon and egg is the mean APC of the lower of 10⁷ CFU/g. The contamination of mackerel is members of the

Vibrionaceae while shrimp including *Staphylococcus aureus*, *Streptococcus* Group D, and members of the Enterobacteriaceae and Vibrionaceae from the bacterial content have effect of increasing the APC value (Table 2). Therefore, considering the location of the sushi restaurant Zone B, has the highest mean APC value equal to 10⁷ CFU/g

while Zone A and C lower of 10⁷ CFU/g. There are several members of bacteria from the sushi restaurant Zone B, of which the Gram-positive cocci (*Planococcus*, *Micrococcus*), NFB (*Acinetobacter*), FGNB (*Brucella*, *Pasteurella*), and Lactic acid bacteria (*Lactobacillus*) assembled with *S. aureus* APC <10⁷ CFU/g (6.85 log CFU/ml).

Table 1: Microbiological quality of sushi

Place/Sample	Salmon (n=12) (log CFU/ml)	Mackerel (n=12) (log CFU/ml)	Shrimp (n=12) (log CFU/ml)	Egg (n=12) (log CFU/ml)	Mean APC (log CFU/ml)
Zone A	6.79 Acceptable	7.20 Unsatisfactory	6.95 Acceptable	6.56 Acceptable	6.94 Acceptable
Zone B	6.48 Acceptable	7.30 Unsatisfactory	7.23 Unsatisfactory	6.94 Acceptable	7.09 Unsatisfactory
Zone C	6.74 Acceptable	6.35 Acceptable	6.62 Acceptable	6.33 Acceptable	6.55 Acceptable
Mean APC (log CFU/ml)	6.69 Acceptable	7.11 Unsatisfactory	7.01 Unsatisfactory	6.68 Acceptable	6.91 Acceptable

Table 2: Bacterial isolated from sushi

Salmon	Mackerel				Shrimp				Egg										
	A	B	C	isolate	A	B	C	isolate	A	B	C	isolate							
Acinetobacter sp.	1	1	0	2	Arcanobacterium haemolyticum	0	1	0	1	A. molluscorum	0	4	0	4	A. shubertii	1	0	1	2
A. hydrophila	1	0	0	1	A. hydrophila	2	0	0	2	A. veronii	0	2	0	2	A. veronii	0	3	0	3
A. veronii	0	5	0	5	A. shubertii	0	0	3	3	A. hydrophila	1	0	0	1	Bacillus sp.	1	0	0	1
Burkholderia cepacia	1	0	0	1	Corynebacterium sp.	1	0	0	1	Bacillus sp.	0	0	4	4	Burkholderia cepacia	0	1	0	1
Legionella sp.	0	1	0	1	Planococcus sp.	0	3	0	3	Brucella sp.	0	3	0	3	Enterobacter agglomerans	2	0	0	2
Listeria monocytogenes	0	1	0	1	V. cholerae	0	0	1	1	E. coli	1	0	0	1	Enterobacter cloacae	0	0	1	1
Micrococcus sp.	0	2	0	2	V. fluvialis	2	0	0	1	Micrococcus sp.	0	3	0	3	Lactobacillus	0	2	2	4
Staphylococcus aureus	1	9	0	10	V. haveyi	0	0	4	4	Shigella sp.	1	4	0	5	Listeria monocytogenes	0	0	1	1
Streptococcus Group D	0	1	0	1	V. mimicus	2	0	0	1	Staphylococcus aureus	0	0	7	7	Shigella	1	0	0	1
Pasteurella sp.	0	6	0	6	V. vulnificus	2	0	0	2	Streptococcus Group D	1	0	2	3	Staphylococcus aureus	0	8	0	8
Pseudomonas aeruginosa	2	0	0	2	Yesinia sp.	0	0	0	0	Peptostreptococcus sp.	0	2	0	2	Pasteurella	0	1	7	8
Proteus mirabilis	1	2	0	3	Yeast	0	0	1	1	Planococcus sp.	0	3	0	3	Pennibacillus	0	0	1	1
Proteus myxofaciens	0	1	0	1	total	10	4	9	23	Pseudomonas aeruginosa	2	0	0	2	Peptostreptococcus	0	1	0	1
V. cholerae	0	0	9	9						Proteus myxofaciens	1	0	0	1	Planococcus	0	2	0	2
V. fluvialis	1	4	0	5						V. cholerae	0	3	5	8	V. cholerae	0	3	0	3
V. haveyi	1	3	2	6						V. fluvialis	1	2	0	3	V. fluvialis	5	1	0	6
V. mimicus	0	5	0	5						V. haveyi	0	0	3	3	V. mimicus	0	1	0	1
V. vulnificus	1	0	0	1						V. mimicus	1	2	0	3	Yeast	0	0	4	4
total	10	42	11	63						V. vulnificus	1	0	0	1	total	10	23	17	50
										Yesinia sp.	0	2	0	2					
										total	10	32	21	63					

Table 3.1: Antibiotic susceptibility testing of bacterial isolates from sushi in Zone A and B

Bacterial	Antibiotic (Clear zone/mm.)															Zone
	Amikacin	Ampicillin	Amoxycylav	Cefoxitin	Ceftazidime	Ceftriaxone	Chloramphenicol	Ciprofloxacin	Cefuroxime	Gentamicin	Piperacillin	Tetracycline	Trimethoprim	Imipenem	Meropenem	
<i>Acinetobacter sp.</i>	S	S	S	R	S	R	S	S	R	S	S	S	S	S	S	A
<i>V. vulnificus</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	A
<i>A. hydrophila</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	A
<i>P. aeruginosa</i>	S	S	S	R	S	R	R	S	R	S	S	S	S	S	S	A
<i>P. aeruginosa</i>	S	R	S	R	S	S	R	S	R	R	S	R	R	S	S	A
<i>P.mirabilis</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	A
<i>V. fluvialis</i>	S	S	S	R	S	S	R	S	R	S	S	R	R	S	S	A
<i>V. mimicus</i>	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	A
<i>A. molluscorum</i>	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>A. veronii</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. cholerae</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. cholerae</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. fluvialis</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. fluvialis</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. mimicus</i>	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	B
<i>V. mimicus</i>	S	S	S	R	R	S	S	S	R	S	S	S	S	S	S	B
<i>P. mirabilis</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	B

Table 3.2: Antibiotic susceptibility testing of bacterial isolates from sushi in Zone C

Bacterial	antibiotic (Clear zone/mm.)															Zone
	Amikacin	Ampicillin	Amoxycylav	Cefoxitin	Ceftazidime	Ceftriaxone	Chloramphenicol	Ciprofloxacin	Cefuroxime	Gentamicin	Piperacillin	Tetracycline	Trimethoprim	Imipenem	Meropenem	
<i>A. schubertii</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>A. schubertii</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>A. schubertii</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C
<i>A. schubertii</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>A. schubertii</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>E. cloacae</i>	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. cholerae</i>	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. cholerae</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. cholerae</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C

<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
<i>V. haveyi</i>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C

Pseudomonas aeruginosa was most antibiotic resistance bacteria isolated from Zone A. They can resist four or five antibiotics including cefoxitin, ceftriaxone, chloramphenicol, cefuroxime, gentamicin, tetracycline and trimethoprim/sulfamethoxazole, which can define as MDR bacteria [17]. *Vibrio fluvialis* was most antibiotic resistance *Vibrio* sp., however, there were not cause disease in human. *V. mimicus* was MDR bacteria and isolated from sushi in Zone B. There was resisted to cefoxitin, ceftriaxone and cefuroxime and can cause disease in human (Table 3.1). Bacterial isolates from Zone C were rarely resistance to antibiotics (Table 3.2). In Thailand, Control of antimicrobial resistance bacteria in fishery products is unawareness. However, high prevalence of MDR *E. coli* is contaminated in fish and seafood located in Korea and Vietnam. It may imply that fishery products are reservoirs for MDR bacteria [18, 19, 20]. *P. aeruginosa* can innate and/or acquire resist to several antimicrobial agents by pumping antibiotic out and adjust permeability of outer membrane [21].

CONCLUSION

Genotyping of antibiotic-resistance genes is necessary to control the virulent strain bacteria in public health concern. *P. aeruginosa* infection is occurred in humans by raw fish consumption and its by-product contact. In addition, colony characteristics of *P. aeruginosa* isolated from fish are closely related to *P. aeruginosa* colony that is causing hospital-acquired pneumonia in humans. Corresponding to previous study, *P. aeruginosa* is a major human pathogen, which is reserved in *Oreochromis niloticus* and *Clarias gariepinus*. *OprL* and *toxA* genes are predominant virulent genes, which are associated to *P. aeruginosa* infection. The *bla*CTX-M, *bla*TEM, and *tetA* genes are the major antibiotic-resistance genes that can resist to cefotaxime, amoxicillin, and tetracycline as MDR *P. aeruginosa* strain. This strain is concerning on public health problems [22]. We were concluded that raw topping sushi, such as raw fish and shrimp were risk of MDR contamination. Genotyping of MDR bacteria from fish and seafood topping sushi should be conduct for public health control on cleanliness food process and food hygiene.

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