Analysis of Fish Commonly Sold in Local Supermarkets Reveals the Presence of Pathogenic and Multidrug-Resistant Bacterial Communities

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ABSTRACT: Fish has been an important source of proteins, essential vitamins, and low saturated fats for centuries. However, improperly handled fish can expose consumers to infectious bacteria, including difficult to treat multidrug-resistant pathogens. With the goal to investigate the existence of disease-causing and antibiotic-resistant bacteria, we examined bacterial communities present on various types of fish purchased from supermarkets in Houston, Texas, USA. The bacterial communities were characterized by selective phenotypic culture methods, 16S ribosomal RNA gene sequencing, and antibiotic susceptibility testing. The results revealed the presence of different bacterial communities on the fish samples examined. The bacterial communities were not significantly different between the supermarkets sampled. The following presumptive human pathogens were isolated on the fish samples: *Escherichia coli* (67%), enterohemorrhagic *E. coli* (31%), *Shigella* and *Salmonella* species (28%), *Listeria* species (29%), and *Staphylococcus aureus* (28%). Drug sensitivity assays showed resistance to commonly prescribed antibiotics ciprofloxacin, gentamicin, and vancomycin. Out of a total of 99 *E. coli* samples tested, 41.4% were resistant to ciprofloxacin, whereas 33.3% were resistant to gentamicin. Of the total of 31 *S. aureus* isolates tested, 87% were resistant to ciprofloxacin, whereas 61.3% were resistant to vancomycin. Moreover, some of the *E. coli* strains were resistant to both ciprofloxacin and gentamicin (28%), whereas 49% of the *S. aureus* isolates were resistant to both ciprofloxacin and vancomycin. These results highlight the prevalence of antimicrobial-resistant foodborne pathogens on fish purchased from the supermarkets and underscore the risk associated with improper handling of fish.

KEYWORDS: Foodborne pathogens, fish microbiology, fish microbiota, 16S ribosomal DNA sequencing, bacterial-contaminated fish

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Introduction

Fish has been an integral part of the human diet for many generations, dating back to over 40 000 years.[1] It is an important source of proteins, essential vitamins, and low saturated fats in the world. Consumption of fish is largely seen as a positive health behavior because fish offers several kinds of health benefits.[2–4] For instance, consuming a diet containing fish (at least) once a week can reduce mortality associated with coronary heart diseases.[2–5] Furthermore, many seafoods contain high amounts of key omega-3 fatty acids, which have been shown to help lower the risk of renal deterioration, breast cancer, and promote plasma membrane remodeling.[6,7]

Consequently, increased fish consumption has become a global trend, with levels almost doubling within the past 40 years.[8,9] Seafood products make up about 13.8% to 16.5% of the total animal protein consumed worldwide and about 1 billion people rely heavily on various seafoods as primary source of animal protein.[9] However, seafoods can also expose consumers to various risks because they serve as habitats to a repertoire of both pathogenic and nonpathogenic bacteria. Foodborne outbreaks and the diseases associated with them threaten public health and place enormous burden on health care systems worldwide. In the United States, more than 9 million cases of foodborne diseases occur annually, with many of them leading to hospitalizations and death.[6,10]

As fish consumption is becoming more and more common to the public, there is a growing interest in understanding the risks associated with it. Traditionally, research in this area has focused on the effects of fish-related chemical contaminants and marine toxins on human health.[2] However, both raw and undercooked fish can expose consumers and handlers to many types of bacteria either from their original environment or their postharvest storage and processing conditions.[11,12] Some of the contaminating bacteria can be opportunistic and pathogenic.[13] In this study, we examined bacterial communities present on the skin of fish commonly sold at local supermarkets to

Table 1. Distribution of the type and number of fish purchased from each store.

STORE	TOTAL NO. OF FISH	FISH TYPE	CONDITION OF FISH ANALYZED	TOTAL NO. OF FISH	STORES
A	14	Barb fish	Whole and gutted	2	B, G
B	13	Bass	Whole and gutted	8	A-H
C	12	Catfish fillet	Fillet	10	A-J
D	14	Cod fillet	Fillet	6	A, D-F, H, I
E	12	Croaker	Whole and gutted	5	A-D, G
F	13	Grouper	Whole and gutted	4	B, C, G, H
G	13	Perch	Whole and gutted	7	A, C-H
H	10	Pompano	Whole and gutted	5	A-D, F
I	7	Red snapper	Whole and gutted	8	A-H
J	4	Salmon fillet	Fillet	10	A-J
		Shrimp	Whole	9	A-I
		Smelt	Whole and gutted	7	A-G
		Swai fillet	Fillet	6	A, D-G, I
		Tilapia	Whole and gutted	7	A-G
		Tilapia fillet	Fillet	10	A-J
		Trout	Whole and gutted	8	A, B, D-F, H-J

All of the fish examined were wild-caught, except tilapia and catfish which were farm-raised.

assess the existence of potential disease-causing and antibiotic-resistant bacteria.

Materials and Methods

Preparation of fish samples

Different species of fish were purchased from 10 randomly selected supermarkets (Table 1) in Houston, Texas, USA, from July to September 2015. Based on initial survey and consultation with store workers, a list of fish commonly purchased from each store was compiled. Consequently, different types of fish (one each) identified to be popular from the list were purchased, in whole, gutted, or filleted. These include salmon, catfish, tilapia, shrimp, bass, red snapper, trout, smelt, perch, cod, swai, croaker, pompano, grouper, and barb fish (Table 1). All of the fish were wild-caught, except tilapia and catfish, which were farm-raised. However, information about the country of origin of each fish was not available. For the fish fillet that required processing, they were degutted and cut at the supermarket according to their standard practices. All of the fish samples were packaged by the store in a sterile transparent ziplock bags, transported to the laboratory on ice, and processed within 2 hours of purchase. Depending on the fish type, the average weight ranged from 2 to 25 g.

To isolate bacteria present on the skin, each fish was carefully rinsed with 30 mL of sterile brain heart infusion (BHI) broth medium (Becton Dickinson, Cockeysville, MD, USA). The rinsed broth from each fish was divided into 2 in 50-mL conical tubes; 15 mL was incubated at 37°C for 24 hours under aerobic conditions in a shaking incubator at 250 rpm. The other 15 mL was incubated at 37°C for 24 hours anaerobically in an atmosphere of 10% H_2, 5% CO_2, and 85% N_2 using a Controlled Atmosphere Anaerobic Chamber (Plas-Labs, Lansing, MI, USA). Both aerobic and anaerobic cultures were incubated at 37°C to encourage growth of human pathogens. Frozen stocks (1 mL) of each culture were stored in 10% dimethyl sulfoxide (DMSO) at −80°C until analyzed. The rest of the culture (14 mL each) was centrifuged at 4000*g* for 10 minutes and the bacterial pellets were stored at −80°C until DNA isolation.

DNA extraction and 16S ribosomal RNA gene sequencing

DNA was isolated from each of the thawed bacterial pellets from the aerobic and anaerobic cultures using the Gene Reagent Pack on the Corbett Life Science X-tractor platform (Qiagen, Valencia, CA, USA). The extraction was performed based on the protocol provided by the manufacturer. The concentration of the extracted DNA was determined using NanoDrop (Thermo Scientific, Wilmington, DE, USA). A control sample was also extracted under similar conditions and

reagents, but without bacterial pellet. To identify the bacterial DNA present, the extracted DNA samples were normalized and analyzed by 16S ribosomal RNA (rRNA) gene sequencing. Sequencing was performed at the Alkek Center for Metagenomics and Microbiome Research (Baylor College of Medicine, Houston, TX, USA). The variable (V4) region of the bacterial 16S rRNA gene was polymerase chain reaction–amplified and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using 2 × 250 bp (base pairs) paired-end. The data were analyzed using the CMMR-16S (v4) analytic pipeline, as described previously.[14–16] The 16S rRNA gene sequences were clustered into operational taxonomic units (OTUs) at a similarity cut-off value of 97% using the UPARSE algorithm.[17] The OTUs were determined by mapping to the SILVA database containing only the 16S V4 region to determine taxonomies.[18]

Isolation of presumptive fish-borne pathogens on selective and differential media

To gain insight into the types of pathogenic bacteria species present on the fish, the frozen stocks of the aerobic culture were further examined on selective and differential media. All of the media used were purchased from Sigma-Aldrich (St. Louis, MO, USA). The media used were MacConkey Agar (for *E. coli* species), sorbitol MacConkey agar (for enterohemorrhagic *E. coli*), xylose lysine deoxycholate agar (for *Shigella* and *Salmonella* species), Oxford agar (for *Listeria* species), and Mannitol Salt Agar (for *Staphylococcus aureus*). A loopful of the frozen culture stocks from each of the fish were streaked on these media and incubated under aerobic conditions at 37°C for 24 to 48 hours. Bacterial growth on each of the selective and differential medium was recorded and enumerated. All of the bacterial colonies from each plate were pooled, cultured in BHI broth overnight at 37°C aerobically, and 1 mL of each frozen in 10% DMSO at −80°C until analyzed, as described below.

Assessment of antibiotic susceptibility

To investigate whether the bacterial colonies isolated on the selective and differential media exhibit antibiotic resistance, antibiotic susceptibility test was performed. Due to the large number of samples, only the *E. coli* isolates selected from the MacConkey and sorbitol MacConkey agar and *S. aureus* isolates from the Mannitol Salt Agar were tested. Two commonly prescribed antibiotics were tested on each bacteria. For *E. coli*, the pooled frozen stocks (described above) were initially cultured on MacConkey agar plates containing ciprofloxacin (4 µg/mL) and gentamicin (16 µg/mL) at the minimum resistance breakpoint concentrations reported in the Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines. For *S. aureus*, the pooled frozen stocks were cultured on Mannitol Salt Agar plates containing ciprofloxacin (4 µg/mL) and vancomycin (16 µg/mL). Growth on each medium was recorded and enumerated. Colonies were selected from each plate and the isolates were identified by conventional biochemical tests (for *E. coli*—glucose broth with Durham tubes, methyl red/Voges-Proskauer, and oxidase tests; for *S. aureus*—blood agar plates and coagulase test).[19] Following confirmation of the isolates by the biochemical tests, the minimum inhibitory concentrations (MICs) of each antibiotic were determined (2 isolates per sample) using Etest (BioMérieux, France). Briefly, overnight bacterial suspensions were adjusted to 0.5 McFarland standards, inoculated on Mueller-Hinton agar, and allowed to dry for 30 minutes. The Etest strips were placed onto the agar surface, incubated aerobically at 37°C for 24 hours, and the MICs read following the manufacturer's instructions. *Klebsiella pneumoniae* ATCC BAA-1705 and *E. coli* ATCC 25922 were used as control.

Data analysis

All the data were analyzed and plotted using GraphPad Prism version 6.07 for Windows (GraphPad Software, San Diego, CA, USA). The Student *t* test was used to compare differences between the samples. In all cases, statistical significance was defined as having a P value of $<.05$.

Results

To isolate and evaluate the types of bacteria present on fish commonly sold in Houston supermarkets, 112 fish samples were examined (Table 1). A total of 15 different species of fish were purchased from 10 different randomly selected supermarkets. The variable region of the 16S rRNA gene was sequenced from each of the bacterial DNA to determine the total bacterial composition and diversity. Approximately, 92% of the raw sequenced reads were mapped, yielding a total of 255 OTUs. For all of the fish examined, the aerobic bacteria had an average OTU count of 23, whereas the anaerobic bacteria had an average of 20 OTUs. The OTUs were not significantly different ($P > .05$) among the supermarkets where the fish samples were purchased.

The 16S rRNA analysis revealed a total of 12 bacterial phyla and 168 distinct genera. Firmicutes and Proteobacteria were the most abundant phyla observed in both aerobic and anaerobic bacteria cultures. The rest of the phyla were (in order of decreasing abundance) Bacteroidetes, Actinobacteria, Fusobacteria, Planctomycetes, Tenericutes, Verrucomicrobia, Euryarchaeota, Acidobacteria, Chloroflexi, and Spirochaetae. The 20 most abundant genera observed are shown in Figure 1. A number of unculturable bacterial genera were also detected (data not shown). The culture conditions may have favored the growth of certain bacteria in some of the fish types. For instance, there were no isolates of *Shewanella* from the aerobic cultures of river barb; however, they were present in the anaerobic cultures. In contrast, no *Lysinibacillus* species were identified in the anaerobic cultures of whole tilapia but were present in some aerobic cultures. Furthermore, incubation of the

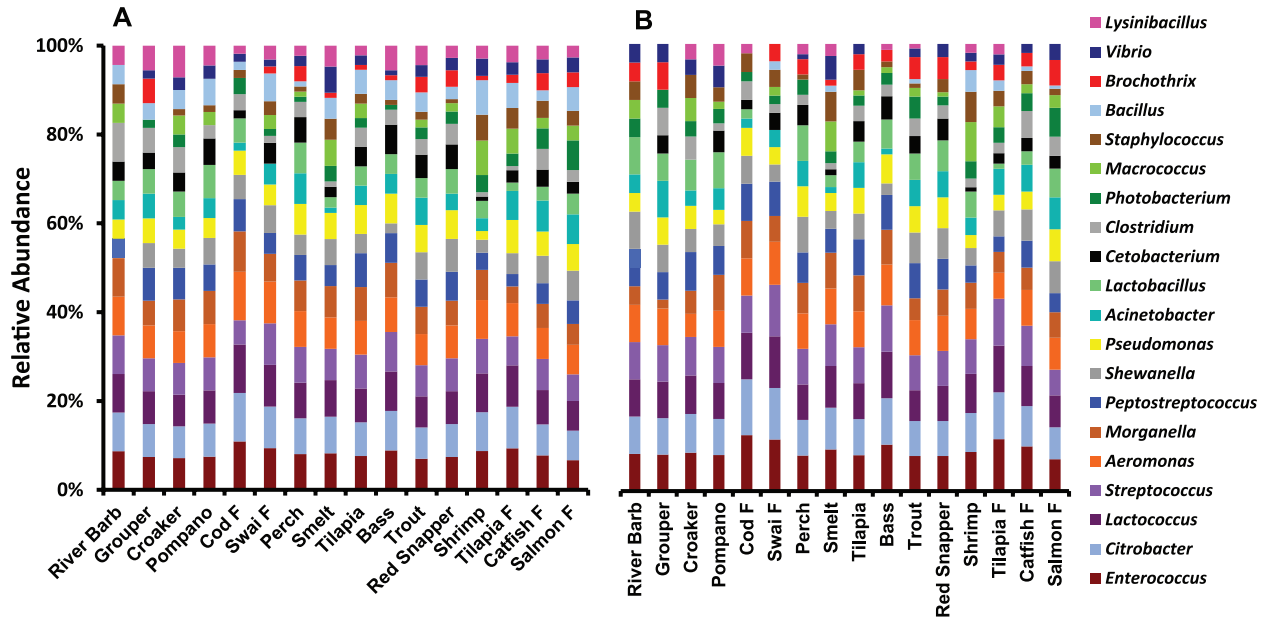

Figure 1. Genera-level relative abundance of the bacterial populations isolated on the fish. DNA isolated from (A) aerobic and (B) anaerobic cultures of bacteria present on the fish was analyzed by 16S rRNA gene sequencing. The V4 region of the bacterial 16S rRNA gene was PCR-amplified and sequenced on the Illumina MiSeq 2×250 bp platform.[14] The 20 most abundant genera out of a total of 168 genera detected are shown. PCR indicates polymerase chain reaction; rRNA, ribosomal RNA.

samples at 37˚C may have also favored bacteria that are adapted to grow at this temperature compared with the bacteria that are adapted to the lower temperatures of their aquatic habitat.

Bacterial genera with both known and unknown pathogenicity

To evaluate whether the bacterial genera identified contain species that are known to cause disease, we used the Pathosystems Resource Integration Center (PATRIC) database[20] and other literature sources to group the genera as pathogenic, nonpathogenic, or unknown pathogenicity. In all of the fish samples examined, bacterial genera with known pathogenic species capable of causing disease were identified (Figure 2-I). The pathogenic potential of the bacterial isolates was further grouped based on supermarkets to assess whether certain bacterial genera may be associated with particular supermarkets. Fish from all of the stores surveyed had bacteria on them that were potentially pathogenic (Figure 2-II). Store H had the highest average number of known pathogenic bacterial genus in both aerobic[12] and anaerobic[10] cultures. Furthermore, store E had the lowest average number[6] of pathogenic anaerobes, whereas store J had the lowest number of pathogenic aerobes.[8] These results suggest random distribution of potential disease-causing bacteria on the fish samples examined, which were independent of the supermarkets from where they were purchased.

To gain insight into the source of bacteria identified, all of the genera associated with pathogenic bacteria were further grouped by their known ecological niche or habitat. About 5% of the pathogenic genera identified could be classified exclusively as soil bacteria, whereas 8% could be grouped as aquatic (Figure 3). Approximately, 35% of the identified genera were widely distributed in nature with no unique ecological niche. Interestingly, most of the genera identified (52%) are known to inhabit various mammalian niches.

The aerobic cultures were further examined on selective and differential media to investigate the presence of pathogenic bacteria commonly implicated in food outbreaks.[21–24] Table 2 shows the number of fish tested and the type of bacteria present. Most of the fish samples had various kinds of presumptive pathogenic bacteria present on them and this was independent of fish type and supermarkets from where they were purchased.

A significant proportion of the bacterial isolates exhibit antibiotic resistance

Due to the presence of pathogenic bacteria on the fish capable of causing human disease, all of the *E. coli* and *S. aureus* isolates (Table 2) were further tested for susceptibility to two commonly prescribed antibiotics. The antibiotics tested were ciprofloxacin (for both *E. coli* and *S. aureus*), gentamicin (*E. coli*), and vancomycin (*S. aureus*). From a total of 99 *E. coli* isolates tested, 41 (41.4%) were resistant to ciprofloxacin, whereas 33 (33.3%) were resistant to gentamicin (Table 3). Out of a total of 31 *S. aureus* isolates tested, 27 (87%) were resistant to ciprofloxacin, whereas 19 (61.3%) were resistant to vancomycin (Table 4). Moreover, some of the *E. coli* isolates were resistant to both ciprofloxacin and gentamicin (28%), whereas a proportion of the *S. aureus* isolates were resistant to both ciprofloxacin and vancomycin (49%). All together, these results indicated that

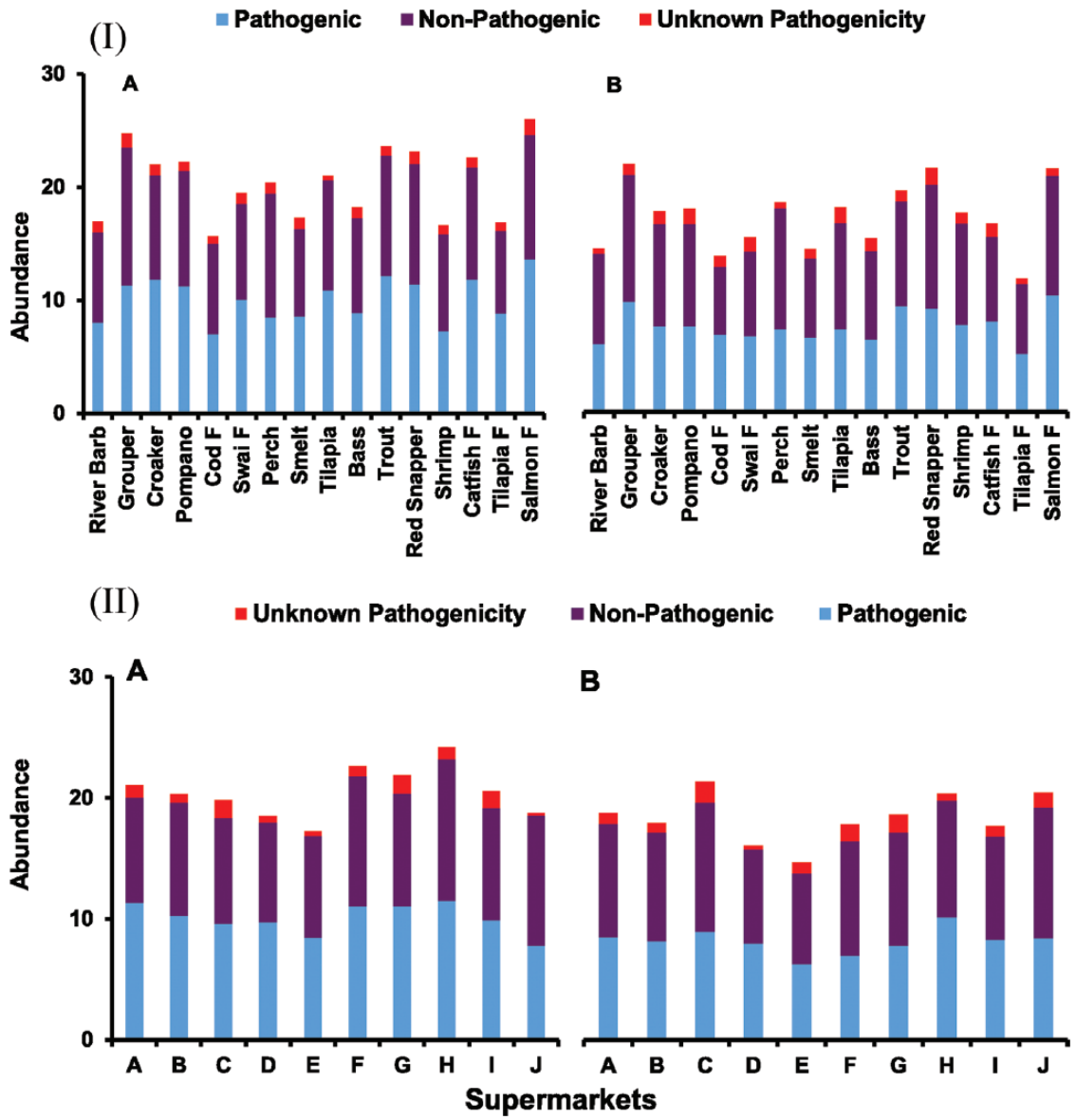

Figure 2. Distribution of bacteria isolated on different fish samples (I) and supermarkets (II) based on pathogenicity. The (A) aerobic and (B) anaerobic bacteria identified to the genera level by 16S rRNA sequencing were grouped into pathogenic, nonpathogenic, and unknown pathogenicity using the PATRIC online database and literature sources.[20] Data represent the mean of the number of genera identified in each group. PATRIC indicates Pathosystems Resource Integration Center; rRNA, ribosomal RNA.

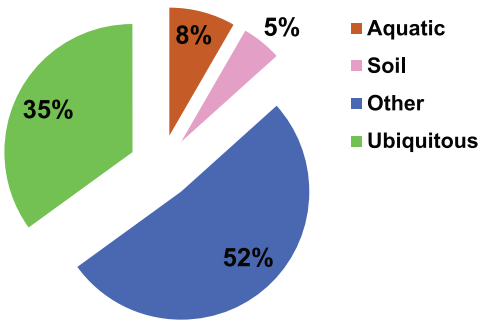

Figure 3. Distribution of bacteria isolated on fish according to their known ecological niche. Bacteria were grouped based on their known ecological niche using literature searches and the PATRIC online database.[20] PATRIC indicates Pathosystems Resource Integration Center.

fish commonly sold in supermarkets could be a major source of pathogenic bacteria capable of causing human diseases and spreading antibiotic-resistant genes.

Discussion

Fish consumption constitutes a major part of the human diet worldwide and provides a variety of rich nutrients key to good health.[18,19] However, pathogens transmitted from fish or the associated aquatic environment can lead to serious infections or even death. Furthermore, modern methods for food production such as minimal processing, mass production, and globalization can facilitate the spread of pathogens.[25,26] Due to the increasing popularity of fish consumption, it has become necessary to study seafood-associated pathogens and their impact on public health. In this study, we examined several fish commonly sold

Table 2. Analysis of the fish-borne bacterial samples on selective and differential media.

TYPE OF FISH	TOTAL NO. OF CULTURE SAMPLES	MACCONKEY	SORBITOL MACCONKEY	XYLOSE LYSINE DEOXYCHOLATE AGAR	OXFORD AGAR	MANNITOL SALT
Salmon fillet	10	7/10	4/10	1/10	4/10	3/10
Catfish fillet	10	6/10	4/10	1/10	2/10	1/10
Shrimp	9	8/9	2/9	4/9	3/9	0/9
Tilapia fillet	10	10/10	1/10	1/10	4/10	0/10
Tilapia	7	5/7	4/7	3/7	2/7	5/7
Bass	8	4/8	2/8	6/8	3/8	2/8
Croaker	5	5/5	3/5	3/5	1/5	3/5
Smelt	7	2/7	1/7	3/7	1/7	1/7
Cod fillet	6	3/6	1/6	0/6	1/6	2/6
Red snapper	8	6/8	2/8	3/8	3/8	2/8
Swai fillet	6	5/6	4/6	2/6	4/6	1/6
Grouper	4	2/4	1/4	0/4	1/4	1/4
Trout	8	5/8	1/8	0/8	2/8	3/8
Perch	7	3/7	4/7	2/7	1/7	0/7
Pompano	5	3/5	1/5	2/5	1/5	5/5
Barb fish	2	1/2	0/2	0/2	0/2	2/2

The media used were MacConkey agar (for *E. coli* species), sorbitol MacConkey agar (for *enterohemorrhagic E. coli*), XLD agar (for *Shigella* and *Salmonella* species), Oxford agar (for *Listeria* species), and Mannitol Salt Agar (for *Staphylococcus aureus*).

at local supermarkets in Houston, Texas, for the presence of pathogens and antibiotic-resistant bacteria using 16S rRNA sequencing and culture methods. Our results revealed a more complex diversity of various bacterial genera than previously reported on fish.[27–29]

The study also revealed the presence of known pathogenic bacteria, including *Aeromonas* species, *Salmonella* species, *E. coli, Listeria monocytogenes, Staphylococcus* species, *Enterococcus* species, and *Vibrio* species on the fish samples analyzed. Some of these pathogens such as *E. coli* are commonly implicated in food outbreaks.[30–33] Interestingly, fish with intact skin had more bacteria present suggesting that the skin probably facilitates bacterial attachment and adherence, which may enable them to colonize. Various anaerobes were also detected even though the samples cultured were from fish skin. This may be due to the presence of aerobic-resistant spores on the fish from contaminating sources or the bacteria were facultative anaerobes. However, the anaerobic cultures were not examined due to the large number of samples tested and will be analyzed in a future study.

The data did not suggest a distinct correlation between the different bacteria identified in the supermarkets. Instead, we observed random distribution of bacteria on the fish examined, which was independent of the supermarkets. Moreover, no correlation in bacteria diversity or abundance between the fish samples that were farmed-raised and those that were wild-caught was found. This indicates that postharvest handling and processing conditions may be involved in facilitating the spread of bacteria. Thus, the source of these pathogens may likely be due to human contamination as they are not part of the known fish skin microbiota.

Further examination of the bacteria isolated from the fish revealed the existence of antibiotic resistance phenotypes. The *E. coli* and *S. aureus* strains were isolated from multiple fish samples that were resistant to ciprofloxacin, gentamicin, and vancomycin, antibiotics commonly prescribed for various bacterial infections. Our investigation is ongoing to identify and characterize the potential sources of bacterial contaminants, antibiotic-resistant genes, and the resistance mechanisms involved. Given that fish harbor multiple bacterial communities living in close proximity to each other, antibiotic resistance in some of these bacteria could lead to easy transfer of resistant genes to others. This could result in increased spread of antibiotic resistance in the human population. The spread of bacteria present on fish with antibiotic-resistant genes may also be exacerbated by consumption of undercooked or raw seafoods. Based on these results, we propose improving sanitary handling and processing of fish to reduce the risk of spread of pathogenic bacteria and pathogens capable of spreading antibiotic-resistant genes in the human population.

Table 3. Ciprofloxacin- and gentamicin-resistance profiles of *Escherichia coli* strains isolated on the fish samples.

TYPE OF FISH SAMPLES	NO. OF CULTURE SAMPLES	CIPROFLOXACIN			GENTAMICIN		
		PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML	PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML
Salmon fillet	10	4/10	8	4-256	2/10	4	16-64
Catfish fillet	10	6/10	12	16-128	4/10	8	8-64
Shrimp	9	4/9	8	4-128	2/9	4	8-32
Tilapia fillet	10	5/10	10	16-128	3/10	6	8-64
Tilapia	7	6/7	12	8- 512	5/7	10	16-32
Bass	6	2/6	4	4-64	1/6	2	16-128
Croaker	5	3/5	6	4-32	2/5	4	32-128
Smelt	3	0/3	0	0	1/3	2	8-128
Cod fillet	4	0/4	0	0	0/4	0	0
Red snapper	8	2/8	4	16-64	3/8	6	16-64
Swai fillet	6	3/6	6	4-128	2/6	4	16-256
Grouper	3	1/3	2	4-16	2/3	4	16-64
Trout	6	1/6	2	8-32	2/6	4	32-128
Perch	7	3/7	6	16-256	1/7	2	16-32
Pompano	4	1/4	2	4-32	2/4	4	16-64
Barb fish	1	0/1	0	0	1/1	2	16-128

Pooled stocks of bacterial isolates that grew on MacConkey agar were further subcultured on MacConkey agar containing ciprofloxacin (4 µg/mL) or gentamicin (8 µg/mL) to select resistant colonies. Following confirmation by biochemical testing, two colonies were selected from each sample and the minimum inhibitory concentrations (MIC) were determined for each antibiotic.

Table 4. Ciprofloxacin- and vancomycin-resistance profiles of *Staphylococcus aureus* strains isolated on the fish samples.

TYPE OF FISH SAMPLES	NO. OF CULTURE SAMPLES	CIPROFLOXACIN			VANCOMYCIN		
		PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML	PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML
Salmon fillet	3	3/3	6	4-128	2/3	4	32-128
Catfish fillet	1	1/1	2	4-32	1/1	2	16-64
Shrimp	0	0	0	0	0	0	0
Tilapia fillet	0	0	0	0	0	0	0
Tilapia	5	4/5	8	8-256	5/5	10	64-256
Bass	2	2/2	4	4-128	1/2	2	32-64
Croaker	3	3/3	6	4-16	2/3	4	64
Smelt	1	1/1	2	32	0	0	0
Cod fillet	2	2/2	4	16-128	0	0	0
Red Snapper	2	2/2	4	16-64	1/2	2	16-32

(Continued)

Table 4. (Continued)

TYPE OF FISH SAMPLES	NO. OF CULTURE SAMPLES	CIPROFLOXACIN			VANCOMYCIN		
		PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML	PROPORTION OF SAMPLES THAT GREW RESISTANT BACTERIA	NO. OF ISOLATES TESTED	MIC, MG/ML
Swai fillet	1	1/1	2	4-64	1/1	2	32-64
Grouper	1	1/1	2	4-16	1/1	2	32
Trout	3	3/3	6	8-64	2/3	4	32-128
Perch	0	0	0	0	0	0	0
Pompano	5	2/5	4	4-16	3/5	6	32-128
Barb fish	2	2/2	4	8-128	1/2	2	32-64

Pooled stocks of bacterial isolates that grew on Mannitol Salt Agar were further subcultured on Mannitol Salt Agar containing ciprofloxacin (4 µg/mL) or vancomycin (16 µg/mL) to select for resistant colonies. Following confirmation by biochemical testing, two colonies were selected from each sample and the minimum inhibitory concentrations (MIC) were determined for each antibiotic.

Author Contributions

AO: Sample collection, performed experiments, data analysis, manuscript writing. DB: Performed experiment, data analysis. MO: Data analysis, manuscript writing. NA: Data analysis. CD: Data analysis, manuscript writing.

REFERENCES

1. Hu Y, Shang H, Tong H, et al. Stable isotope dietary analysis of the Tianyuan 1 early modern human. *Proc Natl Acad Sci USA*. 2009;106:10971–10974.
2. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health. *JAMA*. 2006;296:1885–1889.
3. Iwamoto M, Ayers T, Mahon BE, Swerdlow DL. Epidemiology of seafood-associated infections in the United States. *Clin Microbiol Rev*. 2010;23:399–411.
4. Zheng JS, Hu XJ, Zhao YM, Yang J, Li D. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. *BMJ*. 2013;346:f3706.
5. Bonaccio M, Ruggiero E, Di Castelnuovo A, et al. Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study. *Nutr Metab Cardiovasc Dis*. 2017;27:865–873.
6. Martinović T, Andjelković U, Gajdošik MŠ, Rešetar D, Josić D. Foodborne pathogens and their toxins. *J Proteomics*. 2016;147:226–235.
7. Caires R, Sierra-Valdez FJ, Millet JRM, et al. Omega-3 fatty acids modulate TRPV4 function through plasma membrane remodeling. *Cell Rep*. 2017;21:246–258.
8. Ge C, Lee CS, Yu Z, Lee J. Comparison of bacterial profiles of fish between storage conditions at retails using DGGE and banding pattern analysis: consumer's perspective. *Food Nutr Sci*. 2012;3:190–200.
9. World Health Organization (WHO). *Global and Regional Food Consumption Patterns and Trends*. WHO; 2016. http://www.who.int/nutrition/topics/3_foodconsumption/en/index5.html.
10. Chen Parker C, McKenna C, Wise M, Gezon C, Klontz KC. Geospatial mapping of early cases in multistate foodborne disease outbreaks: a strategy to expedite identification of contaminated imported produce, United States, 2006 to 2013. *J Food Prot*. 2017;80:1821–1831.
11. Balière C, Rincé A, Blanco J, et al. Prevalence and characterization of Shiga toxin-producing and enteropathogenic *Escherichia coli* in shellfish-harvesting areas and their watersheds. *Front Microbiol*. 2015;6:1356.
12. Vázquez-Sánchez D, López-Cabo M, Saá-Ibusquiza P, Rodríguez-Herrera JJ. Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (northwest Spain). *Int J Food Microbiol*. 2012;157:286–296.
13. Sobel J, Painter J. Illnesses caused by marine toxins. *Clin Infect Dis*. 2005;41:1290–1296.
14. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J*. 2012;6:1621–1624.
15. Hasegawa K, Mansbach JM, Ajami NJ, et al. Association of nasopharyngeal microbiota profiles with bronchiolitis severity in infants hospitalised for bronchiolitis. *Eur Respir J*. 2016;48:1329–1339.
16. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335–336.
17. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. 2013;10:996–998.
18. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013;41:D590–D596.
19. Winn WC, Koneman EW. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006.
20. Wattam AR, Abraham D, Dalay O, et al. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res*. 2014;42:D581–D591.
21. Benahmed F, Wang H, Beaubrun JJ, et al. Detection of *Salmonella enterica* subsp. *enterica Serovar Cubana* from naturally contaminated chick feed. *J Food Prot*. 2017;80:1815–1820.
22. Gymoese P, Sorensen G, Litrup E, Olsen JE, Nielsen EM, Torpdahl M. Investigation of outbreaks of *Salmonella enterica Serovar Typhimurium* and its monophasic variants using Whole-Genome Sequencing, Denmark. *Emerg Infect Dis*. 2017;23:1631–1639.
23. Henry MK, Tongue SC, Evans J, et al. British *Escherichia coli O157* in Cattle Study (BECS): to determine the prevalence of E. coli O157 in herds with cattle destined for the food chain. *Epidemiol Infect*. 2017;145:3168–3179.
24. Gould LH, Walsh KA, Vieira AR, et al. Surveillance for foodborne disease outbreaks—United States, 1998-2008. *MMWR Surveill Summ*. 2013;62:1–34.
25. Cebrian G, Sagarzazu N, Pagan R, Condon S, Manas P. Heat and pulsed electric field resistance of pigmented and non-pigmented enterotoxigenic strains of *Staphylococcus aureus* in exponential and stationary phase of growth. *Int J Food Microbiol*. 2007;118:304–311.
26. Abee T, Wouters JA. Microbial stress response in minimal processing. *Int J Food Microbiol*. 1999;50:65–91.
27. Shikongo-Nambabi MNNN. Control of bacterial contamination during marine fish processing. *J Biol Life Sci*. 2012;3:1–17.
28. Chaillou S, Chaulot-Talmon A, Caekebeke H, et al. Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. *ISME J*. 2015;9:1105–1118.
29. Karaaslan A, Soysal A, Kepenekli Kadayifci EDA, et al. *Lactococcus lactis* spp *lactis* infection in infants with chronic diarrhea: two cases report and literature review in children. *J Infect Dev Ctries*. 2016;10:304–307.
30. Awaisheh SS. Survey of *Listeria monocytogenes* and other *Listeria sp.* contamination in different common ready-to-eat food products in Jordan. *Pak J Biol Sci*. 2009;12:1491–1497.
31. Praveen PK, Debnath C, Shekhar S, Dalai N, Ganguly S. Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazards: a review. *Vet World*. 2016;9:6–11.
32. Pagadala S, Parveen S, Rippen T, et al. Prevalence, characterization and sources of *Listeria monocytogenes* in blue crab (*Callinectus sapidus*) meat and blue crab processing plants. *Food Microbiol*. 2012;31:263–270.
33. Momtaz H, Yadollahi S. Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. *Diagn Pathol*. 2013;8:149.