

Full Length Research Paper

Health risk associated with eating fish from brackish water

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Heart disease is a leading cause of morbidity and mortality in the United States with diet being a major contributor. As solution, fish rich in omega-3 fatty acids is recommended. In this study, the impact of brackish water on fish quality, was done by sampling canal water in Edinburg, Texas. Canal water was membrane filtered and cultured on bacteriological media. Pure cultures were phenotypically identified, and isolates confirmed on a BD Phoenix analyzer. The BD Phoenix analyzer, confirmed the following isolates: *Grimontia hollisae*, *Pseudomonas oryzihabitans*, *Pseudomonas pseudoalcaligenes*, *Tatumella ptyseos*, *Pseudomonas fluorescens*, *Klebsiella pneumonia* subsp. *ozaenae*, *Staphylococcus pettenkoferi*, *Pasteurella pneumotropica*, *Achromobacter* species, *Pasteurella aerogenes*, *Moraxella* species and *Pseudomonas* species. Fish consumption is good for maintaining a healthy heart, but consumers risk exposure to foodborne pathogens that can cause watery diarrhea, severe abdominal cramps, nausea, vomiting, fever, bacteremia, urinary tract infections and even dead.

Key words: Brackish, Microbiome, α -Linolenic acid, Linoleic acid, 2,5-diketo-D-gluconic acid, *Tatumellaptyseos*, *Grimontia hollisae*, *Staphylococcus pettenkoferi*, Esoteric, Nosocomial.

INTRODUCTION

Annually, there are about 7,588,000 inpatient cardiovascular surgical interventions in the United States resulting in about 375,295 deaths at a cost of \$320.1 billion (Mozaffarian et al., 2015). A major contributor to this health problem is diets consume by humans today that is rich in omega-6 polyunsaturated fatty (LA) as opposed to pre-industrial revolution era diets that were rich in omega-3 polyunsaturated fatty acid (ALA). This is

because, inside the human body, ALA and LA compete for the metabolic activities of two enzymes identified as delta 6-desaturase and delta 5-desaturase that are found in the smooth endoplasmic reticulum of cells (Burdge and Wootton, 2002; Steffen and Steffen, 2016).

The nature of this competition is such that if the composition ratio of ALA/LA in consumed diet is 1:1 or 1:4 as was the case in pre-industrial revolution era diets

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(Blasbalg et al., 2011; McManus et al., 2011), ALA will preferentially be metabolized through a process of elongation and desaturation (Barceló-Coblijn and Murphy 2009; Russo 2009) to produce eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Burdge and Wootton, 2002). Incidentally, EPA, DPA and DHA are also found in a preformed state in algae, and fatty fish, for which when these food sources are consumed the result is the lowering of bad plasma cholesterol like very low-density lipoproteins (VLDL), low density lipoprotein (LDL) as well as increase in the plasma concentration of good cholesterol like high density lipoprotein (HDL). These activities contribute favorably to making blood less viscous thereby diminishing the prospect of atherosclerotic plaque formation, coronary artery embolism formation as well as lowering cardiac arrhythmia (Connor and Connor, 1997; Simopoulos 2016). These metabolites from ALA when incorporated into the phospholipid bilayer of cell membranes, function to increase fluidity, flexibility and permeability of cells of organ in the body like that of the heart (Arterburn et al., 2006; Kim et al., 2010)

On the contrary, when the dietary composition ratio of ALA/LA in food consumed is 1:15 and greater as is the case in today's Western diet (Connor and Connor, 1997), there is then the preferential metabolization of LA with the resultant formation of arachidonic acid (AA). This long chain polyunsaturated fatty acid functions to elevate bad plasma cholesterol level in the body like very low-density lipoproteins (VLDL), low density lipoprotein (LDL) as well as decrease the plasma level of good cholesterol like high density lipoprotein (HDL) (Connor and Connor, 1997). When AA is incorporated into the phospholipid bilayer of the membranes of cells, it functions to promote pro-inflammatory activities that will cause blood vessel to thicken and constrict platelets to aggregate and form clots (Benatti et al., 2004) that are events that are harmful to the normal physiology of the heart.

Hence, as solution to the high incidence of cardiovascular surgical interventions in the United States of America, there is the need to increase the intake of diet that is rich in the pre-formed omega-3 fatty acids such as EPA, DPA and DHA as opposed to ALA so as to reverse the dietary ratios of polyunsaturated fatty acids in food consumed today from 1:15 to 1:1 or 1:4. Unfortunately, dietary intake of seeds like flaxseed (Ander et al., 2010), nuts like walnuts and common vegetables will only lead to a modest increase of EPA, DPA and DHA in blood plasma and tissue cells (Brenna et al., 2009) because the process of synthesizing EPA, DPA and DHA from ALA in the human body is not very efficient. This is because based on recommendation for an average dietary intake of ALA in the Western diet that is set at 1.3 to 1.7 g/d, only 5% of the consumed ALA gets converted to EPA while only under 0.5% of the ALA

is converted to DHA (Geleijnse et al., 2010). This low conversion rate in comparison to the needs of organs like the heart, brain, liver, and membranes of cells means the human body cannot depend solely on ALA intake to increase the intake of EPA, DPA and DHA (Blondeau et al., 2015). Fortunately, fish and fish oil products as rich source of pre-formed omega-3 fatty acids makes eating fish ideal for increasing EPA, DPA and DHA in the body.

This is why as strategies towards improving the heart health of Americans, some federal government agencies like the Department of Health and Human Services (HHS), the U.S Department of Agriculture and some associations like: the American Heart Association and the American Dietetic Association, recommend that 8 ounce servings of fish be consumed weekly (Bantle et al., 2008; Gebauer et al., 2006). This recommendation is based on epidemiological studies that demonstrate that diets rich in pre-formed omega-3 fatty acid are associated with a healthy heart (Holub 2002; Yancy et al., 2013). But then, increasing fish consumption is not without the risk that consumers may be exposed to foodborne pathogens that thrive in the aquatic environment from where fish are caught. That risk becomes greatest if the aquatic environment from which fish is caught is like the brackish waters of the Rio Grande River that is in the Southern part of Texas.

This river which originates from the south-central part of the State of Colorado, and meanders through the states of New Mexico and Texas before finally emptying into the Gulf of Mexico, is a highly polluted river because raw sewage and sewage treated water from both the Rio Grande Valley in South Texas as well as Northern Mexico are discharged periodically into this river. Also, because the geographical area through which this river flows is sub-tropical in climate, the accompanying arid conditions have resulted in the diversion of the waters of the Rio Grande river to create a network of about 641.9 miles of irrigation canals with 90% of the water dedicated for agricultural use. The presence of these networks of canals has created opportunities for local Anglers to fish in these irrigation canals. Hence, the goal of this study was to analyze water from one of these networks of canals that is identified as the Edinburg North Canal so as to determine its bacterial content as a means to understand if there was any risk to the health of locals who consume fish caught from the waters of these networks of canals.

MATERIALS AND METHODS

Sampling

Water samples from the Edinburg North Canal that is located between Jackson street and McColl street was collected in sterile 500 mL flask s from different sampling locations in the canal. The

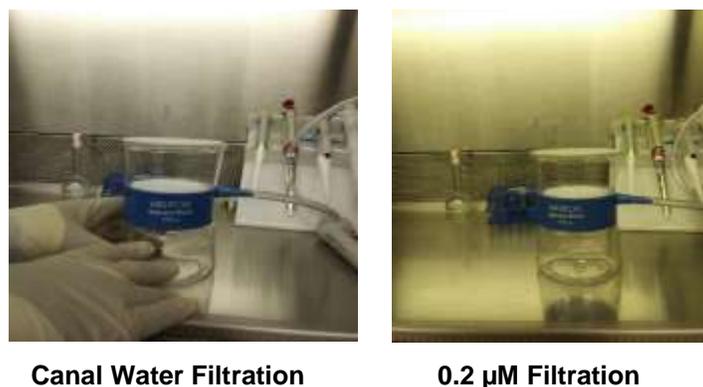


Figure 1. Filtration technique for canal water with Millipore Express Plus filter.



Figure 2. Bacteriological media used for culturing canal water.

samples were properly labelled at the collection site with site name, date, and time of collection prior to samples being transported to the laboratory at the Department of Health and Biomedical Sciences, University of Texas Rio Grande Valley. In the laboratory, the canal water was concentrated by using Millipore Express Plus filters with pore size of $0.22 \mu\text{L}$ (Millipore Corporation, Billerica, Massachusetts) to which a vacuum pump was connected. With a sterile dissecting forceps, the filter paper was removed and placed in Petri dish of Trypticase Soy Agar that was supplemented with 5% sheep blood (TSA II 5% SB), Mannitol Agar MacConkey agar. Plates were incubated at 37°C for 24 h (Figure 1).

Gram stain

An inoculation loop was used to transfer pure bacterial cultures to a drop of physiological saline on a clean glass slide with frosted end. Thin smear was prepared, allowed to air dry, heat fixed and then stained with the Gram stain (Difco Laboratories, Detroit, Mich., USA). The staining steps were crystal violet, 1 min; Gram's iodine (mordant), 1 min; decolorizer, 10-15 s, and safranin, 1 min. Gram's stain of isolates was characterized based on their shape, retention of the primary or secondary stain (Figure 3).

Culture media

The following culture media of: tryptic soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Maryland, USA), MacConkey agar with crystal (BBL Microbiology Systems, Sparks,

Maryland, USA), mannitol salt agar (BBL Sparks, Maryland, USA), xylose lysine desoxycholate, Phenyl ethyl alcohol agar and TSA II 5% SB were removed from a 4°C refrigerator and brought to room temperature. Once acclimatized to conditions suitable for bacteria culture, the plates were then labelled at the bottom part that contain the growth medium with the location from where the water sample was collected and the date of inoculation. Next, with the aid of 10 inoculation loops (0.01), canal water sample that had been concentrated by filtration was plated using the steak plate method on the Sheep blood agar, MacConkey agar, Mannitol salt agar, Phenyl ethyl alcohol agar, Xylose lysine desoxycholate and Tryptic soy agar. The sheep blood agar was used as it represents a general culture medium that will support the growth of fungi as well as Gram positive and Gram-negative organisms. MacConkey and Xylose lysine desoxycholate were used for their support of the growth of Gram-negative organisms while Mannitol salt agar and Phenyl ethyl alcohol agar were used because they support growth of Gram-positive organisms (Figure 2).

Biochemical test

Isolates were subjected to the following biochemical tests: catalase assay was performed by introducing pure bacterial culture from BA (37°C , 24 h) to a drop of 3% H_2O_2 , coagulase test was performed by introducing pure culture of bacteria from BA (37°C , 24 h) to a drop of rabbit plasma, H_2S production was determined by inoculating a pure culture of bacteria on triple iron sugar slants (Difco) and incubated at 37°C for 24 h base as stipulated in the Bergey's Manual of Systemic Bacteriology. A well isolated pure culture was picked from the culture medium using a cotton tip swab.

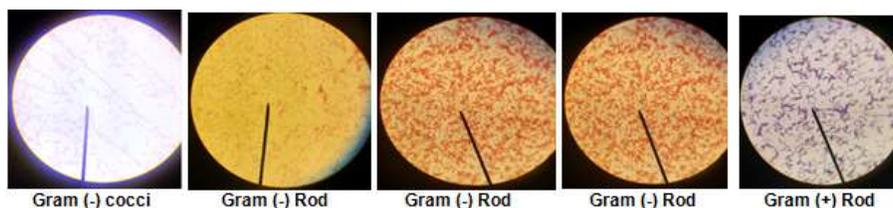


Figure 3. Gram's stain from isolates of pure cultures following incubation at 37°C for 24 h.

A drop of the oxidase reagent, tetramethyl-p-phenylene-diamine was applied to the bacteria cells on the cotton swab and incubated for 30 s to observe for a color change to purple that is a positive oxidase result. Testing for urease was done by inoculating a pure culture of bacteria on a slant of Christensen urea agar slants (Difco) (37°C for 24 h). Urease production was characterized by hydrolysis of urea to form CO₂ and Ammonia resulting in a change in color from orange to pink. A pure culture of test organism was added to broth medium containing nitrate. Bacteria that produce nitrate reductase converted nitrate to nitrite that in the presence of α -naphthylamine and sulfanilic acid will produce a red color (Table 2).

Condition of incubation and duration

Plates with canal water samples that were inoculated by streak plate method in four quadrants were placed in a 37°C incubator for 24 h. After incubation, plates were removed and pure cultures were isolated and transferred to clean slides.

BD Phoenix assay

Brackish water samples were cultured on Trypticase soy agar plates containing 5% sheep blood (TSBA; BD Diagnostics Sparks, MD 21152 USA) for 24 h at 37°C. Gram stain reaction was used to confirm the morphological characteristics of isolates. Using a sterile cotton tip swab, Phoenix ID broth (BD Diagnostics Sparks, MD 21152 USA) was inoculated with pure culture of bacterial colonies. The Phoenix ID tube with the bacteria suspension was vortex and allowed to sit for 10 s. Turbidity of Bacteria in Phoenix ID broth suspension was adjusted to a 0.5 McFarland standard by using a BD PhoenixSpec™ Nephelometer and Standard (BD Diagnostics Sparks MD 21152 USA), according to the manufacturer's recommendations. Integrity of pouch was determined prior to pouch being opened. Phoenix Panel Caddy was removed from pouch and placed on the Phoenix Inoculation Station and the bacteria suspension transferred to panel according to the manufacturer's recommendations. The specimen was logged and loaded onto the instrument within the specified timeline of 30 min. From this point on, the Phoenix Panel Caddy was automatically incubated and results were read at 20-min intervals until the results for all reactions were obtained. A purity control (2 drops of the bacterial suspension) was inoculated onto Trypticase soy agar plates containing 5% sheep blood (TSBA; BD Diagnostics Sparks, MD 21152 USA).

RESULTS

In this study, we were able to identify two major groups of bacteria: the one group like *Pasteurella aerogenes*,

Pasteurella pneumotropica, *Pseudomonas fluorescens*, *Achromobacter* species, *Moraxella* species, *Pseudomonas pseudoalcaligenes* and *Klebsiella pneumonia* subsp. *azaenae* are bacteria that are well established in scientific literatures while another group like *Grimontia hollisae*, *Tatumella pyseos*, *Pseudomonas oryzihabitans* and *Staphylococcus pettenkoferi* are bacteria that are characterized as esoteric (Figure 4). Amongst the isolates in this study that are deemed to be well established in the scientific community is *Achromobacter* spp. that constitutes organisms that can be divided into saccharolytic and asaccharolytic. The asaccharolytic species include *Achromobacter denitrificans*, *Achromobacter piechaudii*, and *Achromobacter faecalis*, while the saccharolytic species is characterized by *Achromobacter xylosoxidans*. For culture characteristics, the organism grew well on MacConkey agar as non-lactose fermenting, on TSA II 5% SB that was supplemented with 5% sheep blood, the organism produced glistening colonies that were flat, spreading, non-hemolytic with edges that were rough. The biochemical capabilities were characterized by positive reactions for catalase, oxidase and citrate and a negative reaction for indole and urease (Table 1). The triple sugar iron agar reaction was alkaline/alkaline with a lack of hydrogen sulfide production.

P. pneumotropica, another isolate that is well established in the scientific community was observed to grow well on TSA II 5% SB as well as on chocolate agar (Figure 2). The culture characteristics on TSA II 5% SB and chocolate agar were of colonies that were convex, smooth, and grayish in color. The biochemical capabilities of this isolate were characterized by a positive reaction for catalase, a positive reaction for oxidase and a variable reaction for urease (Table 2). The organism failed to grow on MacConkey agar even though the Gram stain exhibited Gram-negative rods that were short, straight, and occasionally coccobacillary in shape.

P. aerogenes, grew on chocolate agar and TSA II 5% SB, yielding colonies that were round, grayish in color with smooth or rough edges that were translucent and nonhemolytic Table 3 The organism failed to grow on MacConkey agar. The microscopic morphology of the Gram stain was of cells that stained as Gram negative rods that were cylindrical, short, or straight. The cells

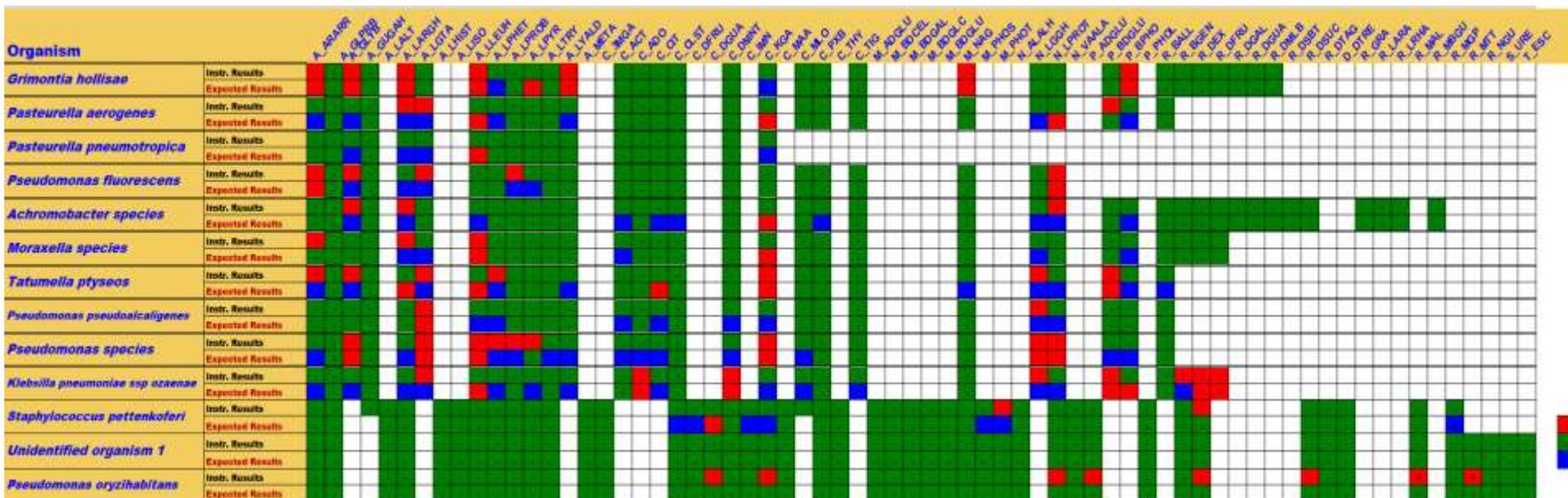


Figure 4. Confirmed bacterial results on the BD Phoenix analyzer. Red denotes a positive biochemical reaction; Green denotes a negative biochemical reaction and Blue denotes an indeterminate reaction.

occasionally appeared as coccobacillary (Figure 3).

Pseudomonas species

The culture characteristics of these isolates were of colonies that were smooth, transparent, and slightly convex on TSA II 5% SB. The microscopic morphology of the Gram stain was of cells that were Gram-negative rods with some cells occasionally exhibiting pointed edges (Figure 3). The biochemical reaction was characterized by a positive catalase, oxidase, citrate, urea hydrolysis and nitrate reduction; a negative indole and hydrogen sulfide production (Table 4).

P. pseudoalcaligenes, displayed a Gram stain microscopic morphology of cells that were cylindrical in shape as well as that stained Gram-negative. The organism grew well on TSA II 5% SB as well as on MacConkey agar and on chocolate agar. The observed culture characteristics were of colonies that were round, smooth, slightly convex, transparent, and non-fluorescent (Table 5).

For *P. fluorescens*, the microscopic morphology of the Gram stain was of cells that were Gram negative rods that were pleomorphic in shape. The biochemical capabilities were characterized by a positive reaction for catalase and oxidase. The culture characteristics on MacConkey agar were of colonies that were non-lactose fermenting

while on TSA II 5% SB, the colonies that grew were glistening, flat, spreading, and non-hemolytic (Table 6).

T. pyseos the first of a group of isolates that are characterized as esoteric displayed microscopic morphology on the Gram stain of cells that were Gram-negative rod. The culture characteristics was of colonies that were non-lactose fermenting on MacConkey agar, while on TSA II 5% SB, the colonies were translucent, glossy, and non-haemolytic. The biochemical characteristics was of cells that failed to utilize citrate as source of carbon, negative reactions for oxidase, indole, and urease as well as a positive reaction for catalase. On triple sugar iron agar medium, the reaction was of Acid/Acid without the production

Table 1. BD Phoenix instrument and expected results for *Achromobacter* species.

Sign	Expected results	Sign	Instrument results
	Glycine	+	Glycine
	L-arginine	+	L-arginine
	L-leucine	-	L-leucine
	Acetate	-	Acetate
	Citrate	-	Citrate
	Colistin	-	Colistin
+	Alpha-ketoglutaric acid	-	Alpha-ketoglutaric acid
	Polymyxin B	-	Polymyxin B
	Gamma-L-glutamyl	-	Gamma-L-glutamyl
	L-proline	+	L-proline

Table 2. Biochemical reaction used for confirming the unique characteristics of isolates.

Bacteria isolate	Biochemical reactions						
	Catalase	Oxidase	Indole	Urea hydrolysis	Citrate	Nitrate Reduction	H ₂ S production
<i>Grimontia hollisae</i>	+	+	+	-	-	+	-
<i>Pasteurella aerogenes</i>	+	+	-	+	-	+	-
<i>Pasteurella pneumotropica</i>	+	+	+	+	-	+	-
<i>Pseudomonas fluorescens</i>	+	+	-	+	+	-	-
<i>Achromobacter</i> species	+	+	-	-	+	+	(v)
<i>Moraxella</i> species	+	+	-	-	-	+	-
<i>Tatumella pyseos</i>	+	-	+	+	+	+	-
<i>Pseudomonas pseudoalcaligenes</i>	+	+	-	-	+	+	+
<i>Pseudomonas</i> species	+	+	-	+	+	+	-
<i>Klebsiella pneumonia ssp ozaenae</i>	+	-	-	-	+	+	-
<i>Staphylococcus pottenkoferi</i>	+	-	-	+	-	+	-
<i>Pseudomonas oryzihabitans</i>	+	-	-	+	+	-	-

of hydrogen sulfide gas. Hence, based on the microscopic morphology and the biochemical reactions observed, this isolate confirmed as a facultative organism that belongs to the family Enterobacteriaceae (Table 7).

G. hollisae, the next esoteric isolate based on the microscopic morphology of the Gram stain, displayed small cells that stained as Gram-negative bacilli. The biochemical capabilities were of cells that were positive for oxidase, indole, and urease reagents. The culture characteristics on TSA II 5% SB were of colonies that exhibited elaborate wrinkling at the edges or better known as rugosity but that failed to grow on MacConkey agar. The rugosity characteristics were accompanied by colonies that produced copious amounts of carbohydrate-enriched extracellular polymeric substances that have been determined to be used for protecting the organism against toxic compounds that are found in brackish water

(Table 8).

S. pettenkoferi, the culture characteristic of this organism on Trypticase Soy Agar with 5% Sheep blood were colonies that appeared circular, opaque glistening with no sign of haemolysis. The microscopic morphology was of cells that stained as Gram-positive cocci in clusters; and the biochemical characteristics were as follows: catalase positive, coagulase negative, urease positive, nitrate reduction positive and H₂S production negative.

P. oryzihabitans, grew on TSA II 5% SB, MacConkey agar and chocolate agar and exhibited colonies that were circular, shiny, smooth, translucent, occasionally wrinkled, and yellow in color. The yellow color and wrinkled colonies were very conspicuous on TSA II 5% SB. The biochemical reactions were as follows: catalase positive, citrate positive, urea hydrolysis positive, oxidase

Table 3. BD Phoenix instrument and expected results for *Pasteurella aerogenes*.

Sign	Expected results	Sign	Instrument results
	arginine-arginine	-	arginine-arginine
	Glycine	-	Glycine
	L-arginine	+	L-arginine
	L-glutamic acid	+	L-glutamic acid
+	L-leucine	-	L-leucine
	L-phenylalanine	-	L-phenylalanine
	Lysine-alanine	-	Lysine-alanine
+	Alpha-ketoglutaric acid	-	Alpha-ketoglutaric acid
	Gamma-L-glutamyl	-	Gamma-L-glutamyl
+	L-proline-NA	-	L-proline-NA
-	PNP-BD-glucoside	+	PNP-BD-glucoside
	Bis (PNP) phosphate	-	Bis (PNP) phosphate

Table 4. BD Phoenix instrument and expected results for *Pseudomonas* species.

Sign	Expected results	Sign	Instrument results
	Arginine-arginine	-	Arginine-arginine
+	Glycine	+	Glycine
	L-arginine	-	L-arginine
+	L-glutamic acid	+	L-glutamic acid
	L-leucine	+	L-leucine
	L-phenylalanine	+	L-phenylalanine
	L-proline	+	L-proline
-	L-pyroglutamic acid	+	L-pyroglutamic acid
	D-mannitol	-	D-mannitol
+	Alpha-ketoglutaric acid	+	Alpha-ketoglutaric acid
+	Gamma-l-glutamyl	+	Gamma-l-glutamyl
+	L-proline	+	L-proline
	PNP-BD-glucoside	-	PNP-BD-glucoside
	Bis (PNP)phosphate	+	Bis (PNP)phosphate

negative, indole negative, nitrate reductase negative, and hydrogen sulfide production negative (Table 2). The microscopic morphology base on the Gram stain was of cells that were small, rod-shaped and Gram negative bacteria.

DISCUSSION

Bacteria associated with fish are usually divided into indigenous and non-indigenous bacteria (Sichewo et al., 2014). The non-indigenous bacteria include *Escherichia coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella* species which are organisms from human and animal waste that have contaminated the aquatic environment of fish. Indigenous bacteria are like *Vibrio* species and *Aeromonas* species that are naturally found

thriving in the aquatic environment of fish (Rodrick, 1991). In this study, because of the polluted nature of the Rio Grande River, that is due to raw sewage and sewage treated water from both the Rio Grande Valley in South Texas as well as from Northern Mexico that are periodically discharged into this river, we had expected that the majority of bacteria will be from human and animal waste. On the contrary, most isolates identified in this study were Gram-negative bacteria that are normal microbiota in domestic animals that are associated with zoonotic infections with only one isolate being Gram positive.

The identified Gram-negative bacteria were: *P. aerogenes*, *P. pneumotropica*, *P. fluorescens*, *Achromobacter* spp., *Moraxella* spp., *P. pseudoalcaligenes* and *K. pneumonia* subsp. *azaenae*. These organisms are familiar to microbiologists because

Table 5. BDPhoenix instrument and expected results for *Pseudomonas pseudoalcaligenes*.

Sign	Expected results	Sign	Instrument results
+	L-glutamic acid	+	L-glutamic acid
	L-leucine	-	L-leucine
	L-phenylalanine	-	L-phenylalanine
	Acetate	-	Acetate
	Citrate	-	Citrate
	D-Mannitol	-	D-Mannitol
	Alpha-ketoglutaric acid	-	Alpha-ketoglutaric acid
	Gamma-L-glutamyl	+	Gamma-L-glutamyl
	L-proline NA	-	L-proline NA

Table 6. BD Phoenix Instrument and Expected results for *Pseudomonas fluorescens*.

Sign	Expected results	Sign	Instrument results
+	arginine-arginine	+	arginine-arginine
	glycine	+	glycine
	L-arginine	-	L-arginine
	L-glutamic acid	+	L-glutamic acid
	L-proline-AMC	+	L-proline-AMC
	L-pyroglutamic acid	-	L-pyroglutamic acid
+	L-proline-NA	+	L-proline-NA
-	Gamma-l-glutamyl	-	Gamma-l-glutamyl

Table 7. BD Phoenix instrument and expected results for *Tatumella ptyseos*.

Sign	Expected result	Sign	Instrument results
	arginine-arginine	+	arginine-arginine
	Glycine	+	Glycine
+	L-arginine	-	L-arginine
	L-glutamic acid	+	L-glutamic acid
+	L-leucine	-	L-leucine
	L-phenylalanine	+	L-phenylalanine
	Lysine-alanine	-	Lysine-alanine
+	Citrate	-	Citrate
+	Alpha-ketoglutaric acid	+	Alpha-ketoglutaric acid
	N-acetyl-galactosamine	-	N-acetyl-galactosamine
	Gamma-L-glutamyl	+	Gamma-L-glutamyl
	L-proline-NA	-	L-proline-NA
+	PNP-BD-glucoside	+	PNP-BD-glucoside
	Bis (PNP) phosphate	-	Bis (PNP) phosphate
	Beta-allose	-	Beta-allose

over the years, by repeatedly processing clinical samples from human infections, policy and procedures were established in laboratory manuals for use to culture these organisms. *P. aerogenes*, is a bacterium that is often

associated with abortion and stillbirth in swine, dogs, rabbits, and infection of wounds in veterinarians, abattoir workers, and animal caretaker workers following bites. It is familiar to Clinical Laboratory Scientist because over

Table 8. BD Phoenix instrument and expected results for *Grimontia hollisae*.

Sign	Expected results	Sign	Instrument results
+	arginine-arginine	+	arginine-arginine
-	Glycine-proline	-	Glycine-proline
+	Glycine	+	Glycine
-	Glutaryl-glycine-arginine	-	Glutaryl-glycine-arginine
+	L-arginine	+	L-arginine
-	L-glutamic acid	-	L-glutamic acid
+	L-leucine	+	L-leucine
	L-phenylalanine	-	L-phenylalanine
+	L-pyroglutamic acid	-	L-pyroglutamic acid
+	Lysine-alanine	+	Lysine-alanine
	Alpha-ketoglutaric acid	-	Alpha-ketoglutaric acid
+	N-acetyl-galactosamine	+	N-acetyl-galactosamine
+	Bis (PNP) phosphate	+	Bis (PNP) phosphate

the years by repeatedly processing specimens to recover this organism from clinical samples, policy and procedure were established for facilitating its recovery. This was also the case with *Achromobacter* spp. which in healthcare facilities, is frequently isolated from dialysis solutions, humidifier solutions, intravenous fluids, and disinfectant solutions like chlorhexidine. In nature, this organism is found in fresh and brackish (Spear et al., 1988) water as well as from municipal and hospital water supply system (Swenson and Sadikot, 2015). Its isolation from sterile solutions in hospitals is because as an opportunistic pathogen the organism is able to contaminate medical devices from immunosuppressed individuals (Duggan et al., 1996) with cardiac diseases, patients with respiratory (Cheron et al., 1994) and gastrointestinal diseases as well as patients with various hematological and solid organ cancers. Once more, by frequently processing clinical samples, the Clinical Laboratory Scientist had established procedures in laboratory manuals for use to facilitate the recovery of this organism.

However, for a small group of Gram-negative bacteria known as: *G. hollisae*, *T. tyseos*, *P. oryzihabitans* and the only Gram-positive bacterium known as *S. pettenkoferi* that were recovered in this study because they were considered esoteric organisms, their work-up was limited to using the services of reference laboratories that had the expertise and capacity for culturing these organisms. The fact that they were associated with clinical samples mostly during an outbreak, Laboratory Directors often preferred to outsource the culturing of these organism to reference laboratories. Which explains why the clinical samples from which *G. hollisae* was isolated were first processed at the Enteric and Special Bacteriology laboratories at the Centers for Disease Control and Prevention. During the initial culture to isolate

this bacterium, it was identified and named *Vibrio hollisae* (Hickman et al., 1982). However, using phylogenetic and phenotypic studies the organism was later re-named as *G. hollisae* (Thompson et al., 2003). The clinical samples sent to CDC were mostly from patients complaining of gastroenteritis with diarrhea as the predominant symptom followed by abdominal cramps, nausea, vomiting, fever, and bloody stools. These patients were individuals living along the Atlantic coast, the Pacific coast and the coastal area of the Gulf of Mexico and were healthy males with a history of having consumed raw seafood like oyster (Hinestrosa et al., 2007).

Moreover, because of repeated outbreaks of a condition characterized as *Vibrio* gastroenteritis in states like Alabama, Florida, Louisiana, and Texas that border the Gulf of Mexico, an initiative was put in place that culminated in the formation of an organization known as the US Gulf of Mexico Regional *Vibrio* Surveillance System (Altekruse et al., 2000). The objective for forming this organization was to facilitate a better understanding of the condition known as *Vibrio* gastroenteritis through a process of research stimulation that will lead to the implementation of strategies that will improve on control and prevention of incidences of the disease especially as infections were sporadic with cases occurring mostly during outbreaks. The success for creating the Surveillance System was that major pathogens involve in causing *Vibrio* gastroenteritis in each of the participating states were identified as well as the frequencies of their infections and are represented as follows: non-O1, non-O139 *Vibrio cholerae* (26%), *Vibrio parahaemolyticus* (25%), *G. hollisae* (14%), and *Vibrio mimicus* (13%) (Hinestrosa et al., 2007; Altekruse et al., 2000). The success of identifying these isolates was leveraged by dependence on the specialized services that were provided by reference laboratories. But then, it will appear

that by focusing on utilizing the services of reference laboratory to culture a bacterium like *G. hollisae* that was considered esoteric this action may have negatively impacted the knowledge and skills of hospital base Clinical Laboratory Scientists. This can be demonstrated by using as an example an incident that occurred at Mount Sinai Hospital in Connecticut that almost had a tragic ending. This is because despite information that *G. hollisae* can be recovered from clinical samples of individuals living along the Atlantic coast of the United States that have consumed raw sea food (Edouard et al., 2009), a patient who was admitted to Mount Sinai Hospital in Connecticut complaining of having abdominal pains and diarrhea for two days following consumption of dried salted fish, had his stool samples cultured only for identification of *Salmonella*, *Shigella* and *Campylobacter* species. Some of the patient's stool samples were examined for ova and parasite to rule-out helminthic infections and for *Clostridium difficile* toxin. With all the microbiological tests being negative, the patient was treated for dehydration and sent home (Rank et al., 1988).

Six days later, the patient was readmitted to the hospital complaining of abdominal pains, vomiting and diarrhea. During this second admission, collected stool samples were cultured using media like TSA II 5% SB, MacConkey agar and Chocolate agar (Figure 2). A 24-h pure culture was suspended in 0.85% physiological saline and inoculated onto an API 20E strip and incubated for 48 h. The use of the API 20E strip resulted in the identification of *G. hollisae* (Rank et al., 1988). Which implies that during the first admission the organism was missed because dependence on the services of reference laboratory has caused the skill and knowledge of clinical laboratory scientists at Mount Sinai Hospital in Connecticut to suffer. Demonstrating the pertinence of updating laboratory manuals annually as well as for Clinical Laboratory Scientists to attend professional organized workshops so as to stay up to date with current or novel isolates like *G. hollisae* which is a vibrio-like organism that cause gastroenteritis, but is not known to grow on thiosulfate-citrate-bile-salts-sucrose (TCBS) (Choi et al., 2012).

Another organism with clinical implications that was identified in this study is known as *T. ptyseos*. Like *G. hollisae*, it is considered esoteric and clinical samples were outsourced to reference laboratory for further processing. Again, like *G. hollisae*, *T. ptyseos* was first isolated at the Enteric and Special Bacteriology laboratories at the Centers for Disease Control and Prevention (CDC) in Atlanta. The genus name of this organism was assigned in honor of a microbiologist who worked at CDC, while the species name is derived from the Greek word *ptyseos* that translates as "a spitting" in English because sputum was the initial clinical sample from which this bacterium was isolated (Hollis et al., 1981). It is a rare food-borne opportunistic pathogen that

has been isolated from fruits like cherries, grapes, and strawberries. Incidentally, *T. ptyseos* is known to be the dominant endosymbionts in the microbiome of a fruit fly known as *Drosophila suzukii* because it feeds exclusively on fresh fruits like cherries, grapes, and strawberries, resulting in *T. ptyseos* being the dominant endosymbiont in its microbiome unlike the case with other fruit flies like *Drosophila melanogaster* that preferentially feed on decaying fruits (Hiebert et al., 2020; Chandler et al., 2014). The feeding habit of *D. suzukii* has been determined to be influenced by attraction to a volatile organic compound (Bueno et al., 2019) known as 2,5-diketo-D-gluconic acid that is produced by *T. ptyseos* (Tracz et al., 2015; Kageyama et al., 1992) and is also responsible for the spread and contamination of other fruits with *T. ptyseos*. Which is why the next time a patient shows up at the hospital complaining of being ill after eating grapes, cherries or strawberries, the Clinical Laboratory Scientist should not only be thinking of setting up cultures to isolate *Escherichia coli*, *Salmonella* species, *Shigella* species or *S. aureus*, media should also be included for isolating *T. ptyseos*.

Furthermore, *T. ptyseos* has been determined to infect other fruits like pineapples in Mexico and in some countries in South America, resulting in a condition known as pink disease. Unfortunately, pineapples infected with *T. ptyseos* that causes pink disease cannot visually be differentiated from those that are un-infected. Determination of infectivity is only possible during the processing phase when slices of pineapples or the juice are pasteurized (Marín-Cevada et al., 2010). This is because when pineapple products are subjected to the technique of pasteurization, a brownish color is produced as a result of a chemical reaction with 2,5-diketo-D-gluconic acid, which is the volatile organic compound that is produced by *T. ptyseos*. This brownish color is what will enable quality control officials know that pineapples have been infected with *T. ptyseos* and will result in the discarding of the processed pineapple products. Unfortunately, this is not the case when whole pineapple fruits are purchased from supermarket stores as there is no way to visually discern if the fruit is infected with *T. ptyseos*. Considering the fact that to the best of our knowledge this is the first case of recovery of this bacterium from canal water that is diverted from the Rio Grande River that demarcates the international boundary between the United States of America and Mexico, this should be a cause for concern especially as *T. ptyseos* has been isolated from pineapples in Mexico that is in close proximity to the Rio Grande Valley where growing of fruits is a dominant part of the economy of the region. *T. ptyseos* has also been isolated from environmental samples like soil in Brazil, water samples in South Africa, dead poultry in Argentina (Agbaje et al., 2011) and from neonatal sepsis in the intensive care unit because contaminated formula milk was fed to neonates

(Mardaneh et al., 2014).

Finally, *S. pettenkoferi* is another esoteric organism that was recovered from samples of canal water from the Rio Grande River. It belongs to a group of bacteria known as coagulase negative *Staphylococcus* (CoNS) that are ubiquitous in nature (Hashi et al., 2015, Huebner and Goldmann 1999) and known to be common colonizers of the skin and mucous membrane of humans and domestic animals (Kaspar et al., 2018). It was first isolated in Germany from blood culture samples of a 25-year-old patient with extra pulmonary tuberculosis and from wound cultures of a 76-year old patient with leukemia, cancer, and insulin-dependent diabetes mellitus (Trülzsch et al., 2002) and was treated as contaminant. The rationale being that in the past, whenever organisms like: *S. aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *E. coli* and other members of the family Enterobacteriaceae were recovered from blood culture, the infection was characterized as true bacteremia and susceptibility testing was immediately done. However, when coagulase negative *Staphylococci* were isolated from blood culture, they were considered contaminant and susceptibility testing was not done. Today, with the increased use of catheters, and arterial lines in patients who are immune-compromised, coagulase negative organisms like *S. pettenkoferi* are no longer treated as contaminant even if only one blood culture bottle in a set is positive. This then explains why these organisms are now the most frequently isolated bacterial species from blood cultures because of contamination from the skin. Coagulase-negative *Staphylococci* to which *S. pettenkoferi* belong, are also known to be involved in infections associated with indwelling catheter and implantable devices. Unlike in the past when this organism was treated as a contaminant today it is classified as an opportunistic pathogen and is increasingly being isolated from other samples like: bone cultures from a 63-year-old diabetic patient who had to undergo a trans tarsal amputation due to osteomyelitis (Loiez et al., 2007), from blood cultures of a 76-year old South Korean man who was suffering from tuberculosis (Song et al., 2009) and in Mexico from blood cultures of a premature baby who survived the infection as well as from blood cultures of an HIV patient with herpes zoster and hepatitis C who later died on the day of admission (Morfin-Otero et al., 2012). In each of these patients, underlying diseases caused them to be immunocompromised thereby creating opportunities for *S. pettenkoferi* to thrive as an opportunistic pathogen.

Conclusion

Meanwhile, just as the intake of omega-3 polyunsaturated fatty acids is based on recommendations associations, consumers should also have the opportunity

from federal government agencies and some to make dietary decisions about increased fish consumption that is based on knowledge about the health of the aquatic environment from which fish is caught. This is significant because the number and types of bacteria in and on fish are directly related to the health of the aquatic environment from which fish thrives. If that aquatic environment has high bacteria count as is the case with polluted brackish water, then fish caught from such an environment will pose a significantly higher health risk to consumers in the way of disease conditions like gastritis, watery diarrhea, severe abdominal cramps, nausea, vomiting, fever, headache, chills and in some cases pneumonia, bacteremia and urinary tract infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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