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Occurrence and Antimicrobial Drug Resistance of Potential Bacterial Pathogens from Shellfish, Including Queen Conchs (Strombus Gigas) and Whelks (Cittarium pica) in Grenada

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Occurrence and Antimicrobial Drug Resistance of Potential Bacterial Pathogens from Shellfish, Including Queen Conchs (Strombus Gigas) and Whelks (Cittarium pica) in Grenada

Abstract

The trade of mollusks and other shellfish play a significant role in the economy of Grenada. The objective of this study was to gather information on the presence of potential human pathogens in clams, oysters, queen conchs, and whelks (West Indian top shell), and to determine the antimicrobial susceptibility patterns of the bacterial isolates. A total of 110 shellfish consisting of 40 clams, 30 oysters, 20 queen conchs, and 20 whelks, was obtained from three different bays along Grenada's coast and examined for bacterial pathogens by culture of whole soft tissue, intestines, feces, and/or meat. Selective media, including thiosulfate-citrate-bile-sucrose agar, were used with the aim to isolate various bacteria, particularly the members of the *Vibrionaceae* family. The isolates obtained were identified based on phenotypic properties, including reactions obtained with the API bacterial identification strips. Of 59 isolates, 35 were identified with >80% probability, with the most prevalent being *Vibrio alginolyticus* (8), followed by *Shewenella putrefaciens* (6), and *Enterobacter sakazakii* (3). Other potential human pathogens included *Vibrio fluvialis* (2), *Stenotrophomonas maltophilia* (2), and *Vibrio parahaemolyticus* (1). All types of shellfish yielded potential human pathogens, including known diarrheal pathogens, *Vibrio fluvialis* from conch and oysters, and *V. parahaemolyticus* from clams. On the antimicrobial drug susceptibility tests for the 35 isolates, using a standard disk diffusion method against 10 antimicrobial drugs, none of the isolates demonstrated resistance to the fluoroquinolone drug enrofloxacin. Rate of resistance among other drugs was highest to ampicillin (60%), and lowest to ciprofloxacin and trimethoprim-sulfa (2.9%). In conclusion, this study revealed the presence of potential human pathogens, as well as antimicrobial drug resistance among bacterial isolates from shellfish in Grenada.
SHORT RUNNING TITLE: Bacterial pathogens from shellfish in Grenada, and their drug resistance
KEY WORDS: Shellfish, bacterial pathogens, drug resistance, Grenada

Introduction

The term "shellfish" encompasses a variety of animals including bivalves – oysters, clams, quahogs, scallops, mussels, and others including crustaceans – lobsters, crabs, and shrimps. Mollusks and other shellfish make up a significant part of natural marine products, and economy of Grenada.

The United States alone imported an average of $3.5 million worth of fish and shellfish between the years 2005 to 2009 (US Census Bureau 2010). Shellfish naturally found in Grenada include: clams (*Mercenaria mercenaria*), oysters (*Isognomon isognomon*), Queen conchs/lambi (*Strombus gigas*), whelks (West Indian top shell) (*Cittarium pica*), and shrimp.

Queen conchs, whelks, and lobster are among the most popular and most consumed shellfish in Grenada. True conchs are marine gastropod mollusks in the family *Strombidae* and the genus *Strombus*, and the locally consumed conch in Grenada is referred to as lambi. Queen conch (*Strombus gigas*) is found throughout the Caribbean (NOAA, 2010), and it is used both for its meat and for its shell. Whelk, known commonly asWest Indian top shell, is a sea snail (Shimek 2005) and it is consumed by many Grenadians. Lobster fisheries are an economically important undertaking in Grenada (McConney et al. 2007) while there have been no efforts to open a hatchery.

Bacteria are ubiquitous in the marine environment. Shellfish can be a source of commensal bacteria that can be pathogenic to shellfish, as well as those pathogenic to humans. There are many bacteria which are pathogenic, but there are some which are more commonly reported. Some of these bacteria include *Salmonella, E. coli, Campylobacter,* and *Vibrio*, and these have been isolated from shellfish and/or the water where the shellfish is found.

Several *Vibrio* species, native to both marine and estuarine environments, have been identified as the causative agents of shellfish-vectored illnesses in humans. These include *Vibrio parahaemolyticus, V. vulnificus, V. cholerae, and V. alginolyticus* (Rippey 1994, Hariharan et al. 1995). Severe *Vibrio* infections are commonly treated with antibiotics, and therefore, antibiotic resistance is a concern. Antibiotics recommended for treatment include tetracycline, ampicillin, and ciprofloxacin (Minnesota Department of Helath 2010). Many bacteria are building resistance through different mechanisms, and antibiotics that were once effective, have had to be replaced. Many foods originating from animals are pretreated with antibiotics, for example, meat from farms, fish and shellfish from hatcheries, etc. Because antibiotics are widely used in the food industry, many of the bacteria are genetically changing and becoming resistant to different antibiotics.

There have been no publications on the microbial hazards posed by shellfish in Grenada. This study was aimed to gather information about the potential bacterial pathogens in shellfish Grenada, and to determine the antimicrobial susceptibility patterns of the bacterial isolates.

Materials and Methods

Geographic areas of sampling, sample collection and processing:

Grenada is a small island measuring 23 x 10 miles, and is located in the West Indies, in the Caribbean. It is the second to the last southernmost island of the Antillean Windward Islands. The specimens chosen for this project were collected from three different bays in Grenada. These included La Sagesse (clams, collected during high tide), Woburn (oysters and whelks), and Calliste (Queen conch/lambi). These three bays are located in the southeast part of Grenada, and they are common places where fishermen collect these shellfish for food.

Forty clams, 30 oysters, 20 queen conchs, and 20 whelks were collected. For processing, each type of shellfish sampled was divided into two equal batches, except for whelks, which were processed as one batch. They were collected in plastic zip-lock bags or in clean plastic bins pretreated with alcohol. The containers were placed in a cooler box with ice packs and transported to the laboratory.

Clams and oysters were checked to ensure there were no abnormalities, and that the shells were closed. They were cleaned externally with tap water and a brush (Whitman and McNair 2004). Oysters were dipped in 70% alcohol. Shells were opened aseptically, and soft tissues were collected with sterile precautions. Pooled tissues were homogenized in a sterile pestle and mortar with 100 grams of sterile sand and 15 ml of peptone water. The homogenate was used as the inoculum for culture.

Queen conchs were collected from Calliste, and brought to St. George's University Marine Station, where they were checked to ensure they were alive and fresh. A hole was made on each between the 3rd and 4th horn ring, and the abductor muscle was severed with a knife. The soft tissue was pulled out and placed in a sterile tray. Fecal material was collected from the gastrointestinal tract, and 2 grams of pooled sample was homogenized with 18 ml of peptone water for inoculum. Samples in duplicate were also collected by swabbing the external surface of the soft tissue, and swabbing a deep incision of the meat, made with a sterile blade.

All twenty whelks were processed immediately in one batch, and were later divided into two batches as non-enriched and enriched samples.

Each whelk was placed on a cutting board. The shell was crushed manually with a pestle making sure that the soft tissue of the whelk did not touch any of the surfaces. Sterile forceps were used to pull the soft tissue off of the shell, and the soft tissue was placed in a sterile pestle, and the pooled sample of 20 whelks was ground with sterile sand and10 ml of peptone water for 5 minutes until one homogeneous mixture was produced, and this was used for culture.

Bacterial Pathogen Isolation and identification:

The process of bacterial pathogen isolation was the same for all samples, which were cultured in duplicate, with and without enrichment. For enrichment, 1 gram of tissue homogenate was added to 9 ml of alkaline peptone water, and incubated at 37?C for 24 hours. Initial inoculations from all samples were made on one plate of each of the following: thiosulfate citrate bile salt sucrose agar (TCBS), blood agar, MacConkey agar, brilliant green sulfa agar (Dico/Becton, Dickinson & Co, Sparks, MD (BD), *Campylobacter* blood-free agar (CBF) (Oxoid Ltd., Basingstoke, Hants, England), and Rappaport broth (Dico/BD). All inoculated media, except for CBF were incubated aerobically at 37°C. Inoculated plates of CBF were placed in jars with 'Campy-Pak' (BD) to create a microaerophilic atmosphere (85% N, 10% CO2, 5% O2).

After 24 hours (48 hours for CBF plates), plates were examined for colonies and their characteristics. Rappaport broth cultures were inoculated onto

Modified Semi-Solid Rappaport Vassiliadis (MSRV) (Dico/BD) media. A Gram stain was performed on each isolated colony from the first inoculation.

If gram-negative curved rods resembling *Vibrio spp.* were observed on TCBS, as described by Farmer et al. (2003), additional tests were performed using gelatin agar, triple sugar iron (TSI) slant, and 0129 vibriostat disc (Oxoid) to help characterize them.

The isolates were then inoculated on to the API 20E strip or other appropriate API system such as API NE, API Staph, and identified as per the manufacturer's (bioMerieux 2010) instructions. The biochemical tests investigated with API 20E were: beta-galactosidase (ONPG), arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), citrate utilization (CIT), H_2S production (H2S), urease (URE), tryptophane deaminose (TDA), indole production (IND), Voges-Proskauer (VP), gelatinase (GEL), utilization of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), saccharose (SAC), melibiose (MEL), amygdalin (AMY), and arabinose (ARA). Cytochrome oxidase test was done separately, and taken into account for reference number completion. API system numbers and percent probabilities in all identification tables were provided by the API system manual (bioMerieux 2010).

Antimicrobial Susceptibility Testing:

A standard disc diffusion method (Jorgensen and Turnidge, 2003) was used. The antibiotics chosen included: ampicillin (AM) (10µg), amoxicillin-clavulanic acid (AMC) (10µg), cephalothin (CF) (30µg), chloramphenicol (C) (30 µg), enrofloxacin (ENO) (5µg), ciprofloxacin (CIP) (5µg), gentamicin (GM) (10µg), streptomycin (S) (10µg), tetracycline (TE) (30µg), and trimethoprim-sulfamethoxazole (SXT) (25µg) (all from BD). The results were interpreted as per Clinical Laboratory Standards Institute (formerly, NCCLS) standards as recommended by Jorgensen and Turnidge (2003), and Becton Dickinson (BD) (2007). For vibrios the interpretive standards for *Enterobacteriaceae* were used, as recommended by Farmer et al. (2003).

Interpretative standards for *Enterobacteriaceae* were used for gram-negative organisms for which no interpretative standards have been described. An *E. coli* strain ATCC 25922 (American Type Culture Collection, Manassas, VA) with known sensitivity patterns was used as a control to test the integrity and activity of the antibiotic discs prior to testing.

Results

None of the samples were positive for *Campylobacter* or *Salmonella*. A total of 59 isolates were recovered from TCBS agar, BG agar, MacConkey agar, or blood agar plates. Of these, 35 isolates were identified with >80% probability (Table 1). Of these, clams yielded 6, oysters and Queen Conch, 8 each, and whelks, 13. The most common species was *Vibrio alginolyticus*, followed by *Shewenella putrefaciens*. Details of isolates from each type of shellfish are given below:

Clams:

Three morphologically different colony types of *Vibrio alginolyticus* were obtained. These were identified with 99.5 %, 99.7%, and 98.5% probabilities. The other isolates were: *Aeromonas salmonicida* (99% probability), *Pantoea* spp. (99.2%), and *Chrysobacterium meningosepticum* (70.4%).

Oysters:

The isolates obtained were, one each of *Escherichia coli* (99.7% probability) and *Vibrio alginolyticus* (99.6%), two isolates of *Shewenella putrefaciens* (99% and 89%), one isolate each of *Moraxella lacunata* (85.7%), *Pantoea* spp (85.7%), *Vibrio fluvialis* (83.9%), and *Stenotrophomonas maltophilia* (80%).

Queen Conchs:

The isolates obtained from Queen conch feces were, one each of *Photobacterium damselae* (99.9%), *Vibrio fluvialis* (98.5%), *Stenotrophomonas maltophilia* (92.4%), and *Klebsiella pneumoniae* ssp. *rhinoscleromatis* (98.6%).

From Queen Conch meat incision swabs, there were 3 isolates of *Enterobacter sakazakii* (98.4%-99.9%). Other isolates were *Vibrio fluvialis, Vibrio alginolyticus*, and *Pantoea* spp. with poor or doubtful profiles (43%-64%).

From Queen conch external meat swab, there was one isolate of *Klebsiella pneumoniae* ssp. *rhinoscleromatis* (98.6% probability), and the remaining were of poor probability (≤ 64%), and included *Shigella* spp., *Pasteurella* spp., *Vibrio alginolyticus*, and *Leifsonia aquatica*.

Whelks:

The bacteria recovered included 4 isolates of *Shewenella putrefaciens* (all 99.9%), one isolate of *Escherichia coli* (92.1%), 4 isolates of *Vibrio alginolyticus* (86%-97.8%), 2 isolates of *Pseudomonas fluorescens* (both 94.1%), one isolate each of *Chrysobacterium meningosepticum* (87.5%), and

Aeromonas hydrophila (85%). Other isolates had profiles ≤ 76%, and included *Staphylococcus epidermidis, Chrysobacterium indologenes*, and *Shigella* spp.

Antimicrobial drug resistance:

There was resistance to nine of the ten antibiotics used (Table 2). Resistance was highest to ampicillin (60%), followed by cephalothin (40%), streptomycin (28.6%), amoxicillin-clavulanic acid (20%), tetracycline (17.1%), chloramphenicol, gentamicin, ciprofloxacin, and trimethprim sulfa (all <10%). All isolates were susceptible to enrofloxacin. Bacterial isolates which were susceptible to every antibiotic included, one isolate each of *V. parahaemolyticus, Escherichia coli,* and *Chryseobacterium meningosepticum* Many isolates were resistant to more than on antibiotic used for susceptibility testing. One isolate of *Stenotrophomonas maltophilia* showed simultaneous resistance to ampicillin, amoxicillin-clavulanic acid, chloramphenicol, cephalothin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. It was susceptible only to fluoroquinolones and gentamicin (Table 3).

Discussion

Oysters, mussels, and other shellfish have a long history of being vectors of infectious agents, especially when they are consumed raw or partially cooked. Many of these infectious agents are native to the marine environment. As filter-feeding organisms, shellfish magnify public health problems associated with environmental contamination because they accumulate microbial pathogens many fold over the densities found in overlying waters (Lee et al. 2003). In terms of the severity of human illness and death, the *Vibrio* genus, specifically *V. vulnificus*, presents a serious problem (Rippey 1994). In a study in the United States (Hood et al.1984), it was observed that certain types of shellfish are more likely to harbor vibrios than others. For example, clams may be far less likely a source of vibrio-related gastrointestinal illness than oysters.

No published information is available regarding the occurrence of bacterial pathogens in various shellfish in Grenada. The present study on shellfish in Grenada, specifically clams, oysters, Queen conch, and whelks, revealed the presence of potential human pathogens, as well as bacteria that may cause morbidity and mortality in shellfish or other marine animals. The organisms isolated in the present study, which are known to have a worldwide distribution in marine animals or their environment, include *Vibrio alginolyticus, V. fluvialis, V. parahaemolyticus, Aeromonas hydrophila, Shewenella putrefaciens, Stenotrophomonas maltophilia, Klebsiella pneumoniae, Pseudomonas fluorescens*, and *Pantoea* spp. (Twedt 1989, Dignani et al. 2003, Buller 2004, Furushita et al. 2005, Pagniez and Berche 2005).

Thirty-five bacterial isolates were identified with >80% acceptable probability, using the API bacterial identification system. The API 20E system, is suitable for identification of *Enterobacteriaceae* and *Vibrionaceae* (Koneman 1992). O'hara et al. (2003) did a study comparing the accuracy of six commercially available systems for identification of members of the family *Vibrionaceae*, and found that 92% of *V. alginolyticus*, 97% of *V. parahaemolyticus*, and 100% of *V. damselae* are correctly identified by API 20E. No system identifies all vibrios correctly by 100%. Boinapali et al. (2007) also used API 20E for identification of vibrios in a study about antibiotic resistance in shrimp in South Carolina. The use of API 20E is recommended for identification of *Vibrio spp.* for practical identification of bacteria from fish and other aquatic animals (Buller 2004). In a recent article published by Okoh and Igbinosa (2010), API 20E was exclusively used to identify marine vibrio strains, consisting of *V. vulnificus, V. metschnikovii, V. fluvialis,* and *V. parahaemolyticus*.

Most of the bacterial isolates identified in this study have been known to cause pathogenicity in humans. None of the isolates were identified as *Vibrio vulnificus*, a major human pathogen. *Vibrio alginolyticus*, which can cause wound infections as well as gastroenteritis, was found in whelks, clams, and oysters, with maximum numbers in whelks. In a study on oysters and mussels in the Netherlands (Schets et al. 2010), 80% of *Vibrio* isolates belonged to *V. alginolyticus* species, and 10% were *V. parahaemolyticus*. *Vibrio alginolyticus* was the most common organism isolated from mussels in Prince Edward Island, Canada (Hariharan et al. 1995). Ripabelli et al. (1999), who studied mussels from Adriatic sea, Italy also observed that vibrios were the most common group isolated, with *V. alginolyticus* as the most common species. No *Salmonella*, or *Campylobacter* was isolated in their study on mussels, as was the case in the present study of shellfish in Grenada. Similarly, Miller et al. (2006) could not detect *Campylobacter* or *Salmonella* in mussels from coastal California. However, oysters sold in Trinidad have been found to be positive for *Salmonella* and *E. coli*, including O157 strains (Rampersad et al. 1999). The single isolate of *V. parahaemolyticus* in the present study originated from

clams. *V. parahemolyticus* is known to cause gastrointestinal illness and has also been isolated from infected wounds (Buller 2004). *V. parahemolyticus* was isolated by Hood et al. (1983) from oysters as well as *V. cholera*, and by Deepanjali et al. (2005) in India. Marine halophilic vibrios related to *V. parahemoliticus* and *V. natrigenes* have been isolated from the coastal waters of Prickly Bay, Grenada in a study by Caputo et al. (2008). *Vibrio fluvialis*, another gastrointestinal pathogen, was isolated from oysters and conch in the present study. *V. fluvialis* has been documented to cause acute gastroenteritis following ingestion of oysters carrying these bacteria (Levine, et al. 1993). *V. fluvialis, V. alginolyticus, and V. parahemolyticus* were all isolated by Maugeri et al. (2000) from brackish waters and mussels in Sicily and Italy.

Among members of the genus *Aeromonas*, *A. hydrophila* is well known for its involvement in gastroenteritis. *A. hydrophila* was isolated from whelks in the present study. Hood et al. (1983) also found *A. hydrophila*, but in oysters, as did Ristori et al. (2007) in a study which identified pathogenic bacteria associated with oysters and estuarine waters in Brazil. In the aforementioned study, *V. vulnificus,* and *V. parahemolyticus* were also identified. *Aeromonas salmonicida* was isolated from clams. This bacterium is mostly a fish pathogen.

Stenotrophomonas maltophilia, formerly known as *Pseudomonas maltophilia* or *Xanthomonas maltophilia,* was isolated from oysters and conches in the present study. It has been known to cause bacteremia and has been involved in wound and soft tissue infections in humans (Gilligan et al. 2003). *S. maltophilia* has also been isolated from water sources and fish (Rodrigues et al. 2003). Nevertheless, little is known about its involvement and its pathogenicity regarding shellfish.

Escherichia coli was isolated from oysters and whelks in the present study. *E. coli* is a well-known human pathogen, and among other systems, pathogenic strains affect the gastrointestinal system most (Edgeworth, 2005). Being a human pathogenic bacterium, *E. coli* is found in water, and has been isolated from shellfish such as clams (Levesque et al. 2006). Previous research in Grenada has shown that *E. coli* is commonly found in the waters of Grenada (Davis et al. 2004, Patel et al. 2010), and this present study demonstrates that shellfish from Grenada can harbor this potential human pathogen.

Enterobacter sakazakii was isolated from conchs. This bacterium has been implicated in causing gastrointestinal disease as well as sepsis. Kim et al. (2008) found this organism commonly in dried shrimp in Korea. Its presence in shellfish of Grenada could be of concern. *E. sakazakii* usually causes symptoms in immunocompromised humans, commonly infants (Anon. 2010).

Pantoea spp. was isolated from clams and oysters. This bacterium has been known to cause wound infections, and gastrointestinal disease (Buller, 2004). Shellfish are not a usual host. Nevertheless, it was isolated from oysters and clams in the present study. Vieira et al. (2004) reported its presence in crabs marketed in Brazil.

Shewanella putrefaciens was isolated from oysters and whelks and *Pseudomonas fluorescens* was isolated only from whelks. Both bacteria are cause for public health concern, since many *Pseudomonas spp.* are becoming more resistant to antibiotics. *S. putrefaciens* has been isolated from human specimens and has been the cause of bacteremia, septicemia, skin and soft tissue infections, and peritonitis (Chen et al. 1997). Although a rare pathogen in humans, bacteremic infections are very severe in nature and cause high morbidity (Brink et al., 1995). *P. fluorescens* has also been known to cause bacteremia in immunocompromised individuals (Hsueh et al., 1998).

Klebsiella pneumonia ssp. rhinoscleromatis was isolated from queen conch. This bacterium is a pathogen of public health concern. As the name suggests, it affects the respiratory system by causing a granulomatous infection termed rhinoscleroma, and may spread to the trachea and larynx (Abbott 2003). A study conducted by Paille et al. (1987), revealed the presence of *K. pneumoniae* in oysters of Louisiana in their survey for fecal coliforms.

Another bacterium isolated from Queen conch included *Photobacterium damsela,* which has been involved in causing secondary wound infection in humans, and vibriosis, systemic disease, and death in fish and shellfish. *Chryseobacterium meningosepticum*, isolated from whelks in this study, has been the cause of meningitis in infants (Buller 2004).

Finally, the bacterium *Moraxella lacunata* was isolated from oysters. This organism, although an unusual pathogen, has been reported as a cause of septicemia, as well as meningitis in humans (Ray and Kar 2006, Pavlatou and Athanasiades 1953).

With regard to the use of antibiotics, non-cholera *Vibrio* infections (enteric as well as systemic), are generally treated with ciprofloxacin or doxycycline (Beers and Berkow 1999). The present study shows susceptibility of all vibrio isolates from shellfish to

these drugs. Halophilic vibrios isolated from seafood can be resistant to β-lactam drugs such as ampicillin and older cephalosporins (Ottaviani et al. 2001). This is also evident from the results of the present study. Non-*Vibrio* isolates with resistance to β-lactam drugs included *Pseudomonas fluorescens*, and *Shewanella putrefaciens*, both of which were sensitive to ciprofloxacin.

One of the multi-drug resistant non-*Vibrio* isolates was *Stenotrophomonas maltophilia*, isolated from conch, and it demonstrated resistance to several drugs, including trimethoprim-sulfa. Although nearly ninety percent of strains of *S. maltophilia* are susceptible to trimethoprim-sulfa, resistant strains are emerging (Falagas et al. 2008). Further studies on this trimethoprim-sulfa resistant strain may be worthwhile. This bacterium, isolated from cultured yellowtail from fish has been reported to be resistant to several beta-lactams including a 3rd generation cephalosporin, ceftazidime (Furushita et al. 2005). This organism is well known for its intrinsic resistance to several drugs that are commonly used against gram-negative infections. The resistance profile of our oyster isolate did not match with that of a typical *S. maltophilia*, our isolate being susceptible to all drugs. Further studies are required to clarify this discrepancy

Although some results were expected, there is novel data which has been discovered in this study and needs future investigation.

In summary, this present study on shellfish in Grenada including clams, oysters, queen conchs, and whelks, revealed the presence of potential human pathogens, and some that could be pathogenic to shellfish themselves. The antimicrobial susceptibility profiles revealed that most of the isolates demonstrated resistance to one or more of the antibiotics chosen for the study. This is significant since it demonstrates that there may be resistant strains of the isolates that are developing in the marine environments of Grenada. Even though minimal or no cases have been attributed to these pathogens, it does not mean that shellfish have not been the vectors for gastrointestinal illness of the population of Grenada. Given that the present study has demonstrated the presence of human pathogens in shellfish, public awareness programs should be implemented to educate the population of Grenada, so far as to report if shellfish has been consumed as part of the clinical history of the patient to better attribute a cause to the diseases, to therefore be able to treat the diseases more efficiently, instead of just blindly treating the patients.

Given that resistance to antibiotics was seen among most of the isolates to one or more antibiotics, if unchecked, it can become a concern in resulting in inefficacy of treatment. In other words, multi-drug resistant human pathogens might be emerging.

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Illustrations

Illustration 1

Tables

Table 1. Bacterial isolates from shellfish identified with >80% acceptable probability using API bacterial identification system

Bacterial isolate	Clams	Oysters	Conch	Whelks	Total no.
Vibrio alginolyticus	3	1	0	4	8
V. parahaemolyticus	1	0	0	0	1
V. fluvialis	0	1	1[a]	0	2
Aeromonas hydrophila	0	0	0	1	1
Stenotrophomonas maltophilia	0	1	1[a]	0	2
Escherichia coli	0	1	0	1	2
Enterobacter sakazakii	0	0	3[b]	0	3
Pantoea spp.	1	1	0	0	2
Aeromonas salmonicida	1	0	0	0	1
Shewanella putrefaciens	0	2	0	4	6
Pseudomonas fluorescens	0	0	0	2	2
Klebsiella pneumoniae	0	0	2	0	2
Photobacterium damsela	0	0	1	0	1
Chryseobacterium meningosepticum	0	0	0	1	1
Moraxella lacunata	0	1	0	0	1
Total	6	8	8	13	35

a feces

b meat incision swab

Table 2. Overall antibiotic resistance of 32 shellfish isolates identified with >80% probability

Antibiotic	**Number Resistant and (R%)**		
	Vibrios No. tested/ N =10	**Other Gram-negative bacteria** N=25	**Total** N=35
Ampicillin	9 (90%)	12 (48%)	21 (60%)
Cephalothin	3 (30%)	11 (44%)	14 (40%)
Streptomycin	4 (40%)	6 (24%)	10 (28.6%)
Amoxicillin-clavulanic acid	2 (20%)	5 (20%)	7 (20%)
Tetracycline	0 (0%)	6 (24%)	6 (17.1%)
Chloramphenicol	0 (0%)	3 (12%)	3 (8.6%)
Gentamicin	1 (10%)	1 (4%)	2 (5.7%)
Ciprofloxacin	0 (0%)	1 (4%)	1 (2.9%)
Trimethoprim-sulfamethoxazole	0 (0%)	1 (4%)	1 (2.9%)
Enrofloxacin	0 (0%)	0 (0%)	0 (0%)

Table 3. Antibiotic Resistance by Bacteria and Antibiotic[1]

Bacterial Isolate & no. of isolates	AM	AMC	C	CF	CIP	GM	S	TE	SXT
Vibrio alginolyticus **(8)**	8	2		3		1	4		
Vibrio fluvialis **(2)**	1								
Vibrio parahaemolyticus **(1)**									
Aeromonas hydrophila **(1)**	1			1			1		
Stenotrophomonas maltophilia **(2)**	1	1	1	1			1	1	1
Enterobacter sakazakii **(3)**				1		1	3		
Escherichia coli **(2)**							1		
Pantoea spp. **(2)**							1		
Aeromonas salmonicida **(1)**				1					
Shewanella putrefaciens **(6)**	4	4		4				4	
Pseudomonas fluorescens **(2)**	2		1	2					
Klebsiella pneumoniae **(2)**	2			1					
Photobacterium damsela **(1)**	1				1				
Chrysobacterium meningosepticum **(1)**									
Moraxella lacunata **(1)**	1		1					1	
Total (35)	**21**	**7**	**3**	**14**	**1**	**2**	**11**	**6**	**1**

[1] All isolates were susceptible to enrofloxacin.

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