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Gastroenteritis Outbreaks Associated with Sapovirus in a Restaurant-Taichung County, 2010

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Abstract

On March 26, 2010, several gastroenteritis outbreaks associated with dining in a restaurant (Restaurant X) in Taichung County were reported to local health bureau. Field Epidemiology Training Program (FETP) of Taiwan Centers for Diseases Control (Taiwan CDC) also received the notification and soon conducted a field investigation. The two confirmed outbreaks (Outbreak A and Outbreak C) occurred on March 10 and March 20, 2010, involving 353 (Attack rate, AA = 69%) and 20 (AA = 91%) people respectively. The major symptoms were diarrhea, abdominal pain, nausea, and vomiting. Fecal specimens from 5 patients in Outbreak A and 3 patients in Outbreak C were positive for sapoviurs, but there was no residual food available for further examination. Among the restaurant workers, fecal specimens from 2 asymptomatic administrative personnel and 1 waitress who used to have gastroenteritis symptoms were also positive for sapovirus. Environmental survey was negative. Based on epidemiological and laboratory studies, sapovirus could be the pathogen in both outbreaks. Infected restaurant employees could result in widespread transmissions, but the definitive transmission route remained to be clarified. Public health officials suspended the restaurant until one week after the last case was found. Infected restaurant workers were asked to stop working. The importance of hand hygiene was emphasized. Temporary kitchens and piping bypassing the original tap water system were used. By April 3, the number of case ceased to increase. In order to respond

INSIDE

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to suspected outbreaks rapidly, public health authorities should be more vigilant on clusters with temporal or geographic associations; health care providers, on the other hand, should report as soon as possible.

Keywords: sapovirus, diarrhea outbreak

Introduction

Sapovirus was first found in Japan in 1977 [1]. Accompanied with norovirus, sapovirus belongs to the *caliciviridae* family; both are important pathogens of human gastroenteritis. Among the five genotypes, all can result in human diseases except genotype III. Because *sapovirus* cannot be cultured in cell lines or animals, electronic microscope [2] and nucleotide sequencing [3] are necessary tools we use to identify its infection. The incubation period for symptomatic infection is 14 to 100 hours and the common symptoms include diarrhea, abdominal pain, vomiting, and fever. Asymptomatic infections have been found [4-5]. Viral load in feces of symptomatic patients usually gradually decreases and becomes undetectable within two weeks, but it has been reported that virus could be detected in feces collected 15 days or 25 days after onset of symptoms. For asymptomatic infection, the pattern of viral shedding, either continuous or intermittent, remains to be determined. In some studies, virus could be identified two weeks after outbreaks [6-7].

Although *sapovirus* was considered to affect infants and young children primarily, especially those in schools or facilities [1, 5, 8-9], outbreaks in adults have been reported more frequently in recent years [4-5, 7]. Fecal-oral transmission is thought to be the major route of transmission, but whether it could be spread by other vehicles is still unknown.

The first outbreak of *sapovirus* -related gastroenteritis in Taiwan was reported in September 2007 [10]; there was no new case found until February 2010. In March 2010, three gastroenteritis outbreaks associated with dining in Restaurant X in Taichung County were reported to local health bureau. FETP of Taiwan CDC also received the notification on March 26, 2010 and soon conducted a field investigation. The purpose of this study was to clarify the extent of these outbreaks and the associations between different outbreaks, to identify the pathogens and routes of transmission, and to offer recommendations on control and preventive measures.

Methods

A. Epidemiological study

A case was defined as a person who developed acute onset of any gastroenteritis symptoms, including diarrhea (stool passage for more than 3 times in 24 hours), vomiting, and abdominal pain, after consuming food from Restaurant X. Because the first outbreak (Outbreak A) occurred among employees of Hospital X after a staff banquet, a retrospective cohort study was conducted by the infection control department of this hospital. Self-administered questionnaires were given to all staff members to identify the case patients, demographics, clinical presentations, and consumed food items. Associations between individual food item and diseases were analyzed. Active case finding through phone interview according to the booking records of Restaurant X was used to identify case in the second and third outbreaks (Outbreak B and Outbreak C). Food items ordered in different outbreaks were compared. For restaurant workers, food handlers were first evaluated on March 15 by local health bureau to see if anyone had gastroenteritis symptoms. Fecal specimen, rectal swab, and nasal swab were taken from 5 workers who were in charge of the banquet. Another evaluation conducted by FETP was done after Outbreak C; self-administered questionnaires were given to all 34 restaurant workers to see if any worker or his/her roommate had gastroenteritis symptoms during February 25 (two weeks prior to Outbreak A) – March 31. Job titles and demographics were also documented. Fresh stools were sampled from all restaurant workers.

B. Laboratory examinations

Nasal and rectal swabs from patients and restaurant workers were sent to identify *Salmonellae*, *E. coli*, *Bacillus cereus*, and *Staphylococcus aureu*s. Fresh stools were sent to identify norovirus by reverse transcription- polymerase chain reaction (RT-PCR) and viral cultures.

C. Environmental survey

On March 27, public health officials from local health bureau, the Third branch of Taiwan CDC, and FETP went to Restaurant X for environmental survey. We assessed the cleanliness of the kitchen, water used to cook, food preservation methods, and food handling practices. Environmental samples were also taken. Working schedule of the restaurant staff was obtained to compare with the time outbreaks occurred.

Results

A. Description of the outbreaks

During March 10 – March 26, three suspected outbreaks of gastroenteritis were reported to local health bureau. Outbreak A occurred after a staff banquet of Hospital X in the evening on March 10; 504 of the 789 workers attended that dinner. The number of patients by day was shown in Figure. Outbreak B occurred after a labor union lunch party on March 20. Because all patients in this outbreak had onset of symptoms before attending the lunch party, Outbreak B was not included in further analysis.

Outbreak C occurred after a private dinner party on March 20, with 22 attendees. The number of cases, major symptoms, attack rates, and results of laboratory examinations were shown in Table 1.

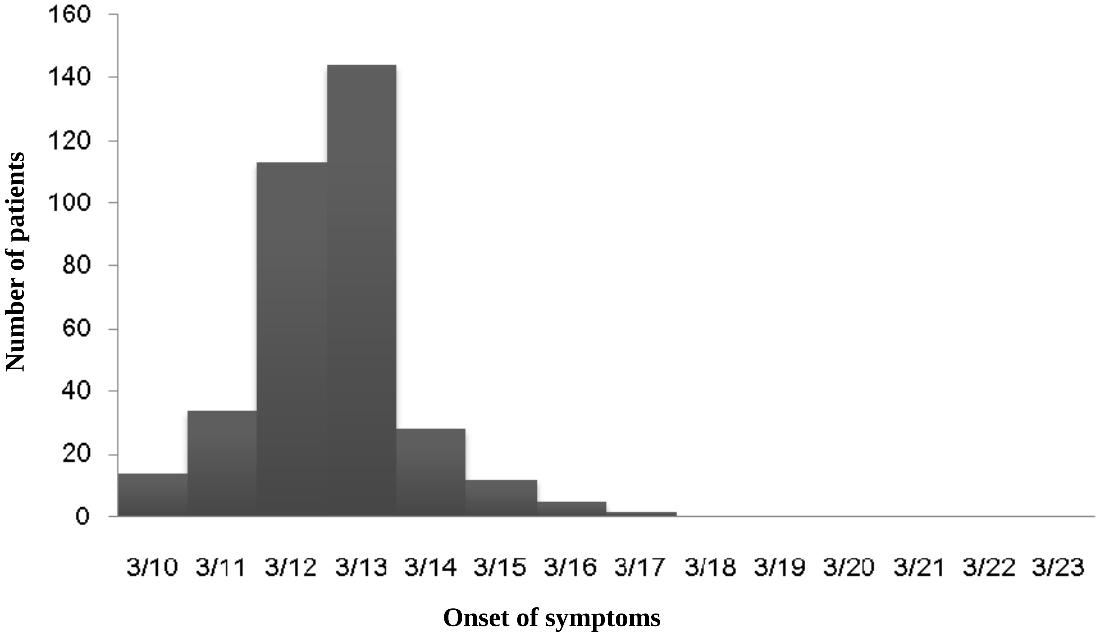

Figure. Number of patients in Outbreak A, by onset date

Table 1. Characteristics and laboratory examination results in Outbreak A and Outbreak C

	Outbreak A	Outbreak C
Dining time	March 10, dinner	March 20, dinner
The date local health bureau received the notification	March 15	March 26
The date Taiwan CDC received the notification	March 26	March 26
Number of attendees	504	22
Number of case-patients	346	20
Median duration from eating to onset of symptoms	50 hours	27 hours
Attack rate	69%	91%
Major symptoms		
Diarrhea	92%	100%
Abdominal pain	73%	-
Nausea	38%	45%
Vomiting	24%	35%
Fever ≥ 38˚C	18%	-
Laboratory results		-
Case- patients	5 positive for sapovirus	3 positive for sapovirus
Restaurant workers	All (5/5) negative	3/34 positive for sapovirus - 2 asymptomatic administrators - 1 waitress who had symptoms

B. Analysis of pathogens

In Outbreak A, 51 fresh fecal specimens from patients were sent to laboratory of Hospital X for bacterial culture; 10 were sent to Research and Diagnostic Center of Taiwan CDC for *norovirus* identification. One patient's stool was positive for *Staphylococcus aureus*; all specimens were negative for *norovirus*. The 10 fecal specimens tested for norovirus were re-examined, and *sapovirus* was detected in 5 by using RT-PCR. In Outbreak C, fecal specimens were sampled from 6 patients, and 3 of them were positive for *sapovirus*. *Sapovirus* was considered to be the etiologic pathogen in these two outbreaks.

In terms of the source of infection and the vehicle of transmission, foods, restaurant workers, and restaurant environments were analyzed.

In Hospital X, consuming food items from Restaurant X on March 10 was significantly associated with illness, but none of the individual food item was associated with increased risk. The attack rate was 76% in vegetarians, similar to that in non-vegetarians. Vegetables, fruit, and dessert were the only identical food items in vegetarians and non-vegetarians (Table 2).

Compared the foods ordered in Outbreak A and Outbreak C, the only common item was Japanese cold platter, but the composition and ingredients in the platter were different. Because the duration between dining and reporting was more than 3 days in both outbreaks, residual food was unavailable for examinations. There was no available evidence supporting that any item of food could be the source of infection or vehicle of transmission.

In addition to 34 principal employees, including 16 direct food handlers dealing with raw food, Japanese food, stir-fried food, and dessert, and 18 non-food handlers, there were 12 temporary workers. Instead of helping each others, food handlers carried out their own duties. Each waiter or waitress was designated to serve 5 specific tables, but they sometimes covered one another. Administrative staff, including accountants, general officer, executive manager, and the owner of the restaurant, rarely dealt with foods. Temporary workers received calls when the restaurant had banquets and worked as attendants.

In Outbreak A, all principal employees denied gastroenteritis symptoms initially and

Table 2. Consumed food items from Restaurant X in Outbreak A

Food item	Number of person who consumed the food	Number of patients (%)
Vegetarians	17	13 (76%)
Japanese cold platter	349	272 (78%)
Thick soup	352	274 (78%)
Prawn	331	256 (77%)
Steamed fish	342	269 (79%)
Braised pork	328	254 (77%)
Rice pudding	327	257 (79%)
Stir-fried seafood	325	254 (78%)
Samgyetang	321	256 (80%)
Herbal & milk paste	296	232 (78%)
Fruits	248	191 (77%)
Green tea	302	238 (79%)

all samples obtained from the 5 direct food-handlers were all negative except one nasal swab which was positive for *Bacillus cereus*. In Outbreak C, although all employees denied gastroenteritis symptoms within 7 days prior to the event, 3 waitresses confessed to have diarrhea and abdominal discomforts within 3 days prior to Outbreak A. None of them could memorize the tables they served on March 10. Twenty-six fecal specimens were sampled and 3 of them (2 asymptomatic administrators and 1 waitress who had symptoms 5 weeks ago) were positive for *sapovirus*.

There were 90 tables in Restaurant X, offering Chinese foods in feasts or buffet. Raw food material was purchased by the owner of the restaurant and preserved in specific refrigerators. Although without concrete partitions, separate areas were used to clean used dishes and manage raw food and cooked food in the kitchen. To maintain hand hygiene, hand sanitizers and disposable gloves were placed at the kitchen and soaps were set at basins. Restaurant workers and customers used the same toilets. Restaurant used tap water to manage food in the kitchen and to flush the toilet; ground water was used to watering the plants. Sick restaurant workers were asked to put on surgical mask or stop working. Comprehensive disinfection with bleach was done on March 15 (5 days after Outbreak A) and March 24 (4 days after Outbreak C).

On the day of environmental survey (March 27), there was no major defect except damaged floor and unclean ice ladle. Samples were taken from raw food shelf, cutting board, and ice from freezer. Only *E. coli* (O8) was isolated from ice; there was no detectable virus in all environmental specimens.

C. Summary of the results

According to the current epidemiological and laboratory evidences, sapovirus was detected in patients from both outbreaks and was considered to be the etiology. Three restaurant workers were found to have gastroenteritis symptoms within 3 days prior to Outbreak A and were the possible source of infection. The two asymptomatic restaurant workers with prolonged viral shedding also could be the transmission source, but whether contaminated food, water or restaurant environment was part of the transmission route was unknown.

Control and preventive measures

Public health officials recommended not to provide raw food in meals and signed a penalty notice with a deadline for the restaurant to improve their environment and food handling practices. Because infected restaurant workers could be the source of infection, hand hygiene was emphasized. Because alcohol hand sanitizer was ineffective in dealing with sapovirus, washing hands with soap before cooking and after toilet use was advised. Sick restaurant workers were asked to stop working. Working gloves should be changed frequently. The local health bureau suspended the restaurant until one week after the last case was found. Temporary kitchens and piping bypassing the original tap water system were used. By April 3, the number of case ceased to increase.

In order to respond to suspected outbreaks rapidly, public health authorities should be more vigilant on clusters with temporal or geographic associations; health care providers, on the other hand, should report as soon as possible. Environmental samples should be taken to clarify the source of infection and casual relationship between pathogens and diseases. An information-sharing platform might be helpful in outbreak investigation. Health care providers, like Hospital X in Outbreak A, should report suspected outbreak as soon as possible.

Discussion

This article described two gastroenteritis outbreaks occurred in March 2010.

In Outbreak A, because the incubation period was compatible with viral gastroenteritis rather than bacteria infection or toxin-related food poisoning, the laboratory personnel kept trying to detect possible etiologic virus despite all fecal specimens were negative for *norovirus* initially. *Sapovirus* was therefore identified. Because all 346 symptomatic hospital workers were banquet attendees and the epidemiologic curve was consistent with common source exposure, the source of infection could be restaurant foods, employees, or environment. Three restaurant workers confessed to have diarrhea within 3 days prior to Outbreak A but did not stop working. However, because only food handlers were sampled, the source of infection could not be confirmed at the first time. Although there was no literature supporting that contaminated food or water could be a transmission vehicle, *sapovirus* has been detected in untreated water, clam and oyster in Japan [11-12] and should not be neglected. In addition, patients in Outbreak A came from many different tables, exceeding the extent one waitress could serve. Widespread transmission from contaminated food or water is of a higher probability despite there was no residual food available for further examination. The three workers who did not stop working could be the cause of the subsequent events.

Because of Outbreak C, a potential source of infection was suspected and an investigation was conducted, samples from all restaurant workers and environments were taken. Among the three specimens positive for sapovirus, two were asymptomatic administrative staff who rarely dealt with foods but may contaminate the environment and lead to outbreak in guests. Hand hygiene is important, not only in food handlers.

Some sapovirus-related food-borne outbreaks have been documented, but the virus has never been identified in suspected food or environment. These two outbreaks were the only two sapovirus-related outbreaks in Taiwan following the first one in May 2007 [10]. But because sapovirus detection is not a regular examination, outbreaks between 2007 and 2010 could not be excluded. The combination of comprehensive field investigation, vigilance of local public health workers, and extensive laboratory studies could help us to respond rapidly.

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Distribution of Pathogenic *Vibrio spp*. in Ocean Areas near Harbors in Taiwan, 2009

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Abstract

Vibrio spp. is naturally found in sea water and estuary. Taiwan is located in subtropical area and surrounded by ocean, which is suitable for growth of *Vibrio spp.* National Quarantine Service (one of the antecedents of Taiwan Centers for Disease Control) proceeded an investigation from 1991 to 1998 and revealed that *Vibrio cholerae* existed in all coastal harbors, including pathogenic (non-O1, non-139) and non-pathogenic serotypes (O1). *Vibrio* *parahaemolyticus* was also noted. Extensive environmental investigation of these pathogens is necessary in order to update the database established a decade ago. In this study, samples were collected from 17 harbors located in northern, central, southern and eastern Taiwan areas. From 204 collected samples, 476 *Vibrio spp.* were isolated. In which, *Vibrio parahaemolyticus* was the most commonly found strain (181 strains, 38%), followed by *Vibrio alginolyticus* (154 strains, 32.4%), *Vibrio vulnificus* (94 strains, 19.7%), *Vibrio metschnikovii* (21 strains, 4.4%), *Vibrio fluvialis* (18 strains, 3.8%) and *Aeromonas sobria* (7 strains, 1.5%). No *Vibrio cholerae* was isolated. The results indicated that the isolation rate of *Vibrio parahaemolyticus and Vibrio vulnificus* was higher than 10 years ago. On the contrary, the *Vibrio cholerae* used to have high isolation rate was not found in this investigation. There was no statistical difference in isolation of each *Vibrio spp.* in all areas. However, there was statistical difference in the isolation of *Vibrio parahaemolyticus, Vibrio vulnificus and Vibrio metschnikovii* in different seasons. Furthermore, statistical difference was also noted in isolation of *Vibrio vulnificus* comparing temperature and salinity. We recommend authorities of public health, environmental protection and fishery should monitor the environmental risk of *Vibrio spp.* in coastal areas around Taiwan regularly, and general public should be informed on the related information. Moreover, database of environmental, clinical bacterial strains, bacterial gene mapping and drug resistance should be established and compared with related data from overseas. This investigation updated environmental monitoring data and also provided a reference for disease prevention and food hygiene policy.

Keywords: harbor, *Vibrio spp*., investigation

Introduction

Vibrio spp. are Gram negative, curved, rod-shaped bacteria [1] which naturally found in sea water and estuary areas. Most of *Vibrio spp.* is halophytic. The amount of *Vibrio spp.* in the environment changes with seasons. In summer (water temperature > 20^{O}C), it is easily isolated from sea water, plankton, algae, bottom mud, fish and shellfish. In winter, the amount of this bacterium evidently decreased but still can be isolated from bottom mud, and not affected by fecal contamination of *E.coli* [2]. This pathogen is mainly found in coastal areas of Gulf of Mexico, South America, Asia, and Australia. Raw or not thoroughly cooked food, or exposure of wound to fish or sea water are the main infection routes, which is mostly seen in summer [3]. *Vibrio parahaemolyticus, Vibrio vulnificus and Vibrio cholerae* are 3 common pathogenic *Vibrio spp.* Clinical signs are related to infected pathogen. Gastroenteritis, wound infection and septicemia are 3 main clinical signs in *Vibrio spp.* infection. Patients with hepatic diseases (hepatic cirrhosis, hemochromatosis, etc.), diabetes mellitus, adrenal gland insufficiency or immune suppressive diseases have higher risk of septicemia [2-3]. The mortality could be 50% in patients with hypotension after arrived hospital; most patients infected by *Vibrio vulnificus* pass away within 48 hours due to fast progressing in disease course [3-4].

In previous survey, conducted by National Quarantine Service from 1991 to 1994 [5] and 1994 to 1998, 2,361 *Vibrio spp.* strains were isolated. The most commonly found species were

Vibrio alginolyticus (910 strains), *Vibrio parahaemolyticus* (451 strains), *Aeromonas hydrophila* (326 strains), *Vibrio cholerae* (201 strains), *Aeromonas sobria* (116 strains), *and Vibrio vulnificus* (114 strains). These 6 main isolated species were accounted for 89.7% of all isolated species. In 2004, study on distribution of pathogenic *Vibrio spp.* in coastal water in Taiwan was conducted by National Science Council. Samples were collected from 6 estuaries and analyzed for *Vibrio parahaemolyticus* and *Vibrio cholerae.* The results found these 2 pathogenic bacteria were present in northern Taiwan but not in central and southern Taiwan [6]. The difference was probably due to different water quality index between these regions and further study was needed.

Taiwan is located in subtropical area and ocean surrounded, which is suitable for growth of *Vibrio spp.* The environmental monitoring project conducted by National Quarantine Service had been postponed for over 10 years. In order to update database of environmental monitoring and to provide references for future regular monitoring studies (every 3 to 5 years), this investigation was proceeded..

Materials and Methods

A. Sample collection:

1. Collection sites: All 8 of category 1 harbors (classified by Council of Agriculture, Executive Yuan) and 9 category 2 harbors were selected. Category 1 harbors include Cheng Pin fishery harbor (Keelung City), Pa Tou Tzu fishery harbor (Keelung City), Nan Fang Ao fishery harbor (Yilan County), Wu Shih fishery harbor (Yilan County), Hsin Chu fishery harbor (Hsinchu City), Wu Chi fishery harbor (Taichung County), Chien Chen fishery harbor (Kaohsiung City), and Yen Pu fishery harbor (Pingtung Couny). Category 2 harbors include Tan Shui fishery harbor (Taipei County), Chu Wei fishery harbor (Taoyuen County), Wang Kung fishery harbor (Changhua County), Pu Tai fishery harbor (Chiayi County), Hsin Ta fishery harbor (Kaohsiung County), Fang Liao fishery harbor (Pingtung County), Cheng Kung fishery harbor (Taitung County), Po Tsai Liao fishery harbor (Yunlin County), and General Ma Sha Kou fishery harbor (Tainan County).
2. Collection time: Four collecting operations were conducted in 2009, i.e., February 9 to March 3; May 4 to May 19; August 3 to September 14; and November 2 to 24.
3. Sampling method: Three collecting sites in each harbor were selected. 450 ml of sea water from 1 meter under sea surface was collected into a 500 ml sterile sampling bottle.
4. Physical measurements: Sea water temperature (by alcohol thermometer), salinity (by SA10T) and pH value (by TECPEL pH703) were measured and recorded.

B. Laboratory examinations:

1. Bacterial counting: 0.2 ml of sample was streaked on TCBS culture agar plate and incubated at 37^{O}C for overnight, and bacterial colony was counted the next day.
2. Bacterial isolation and identification:

a. 20 ml of 10X Alkaline peptone water (pH 9.0 - 9.2) was added into 180 ml sample water and mixed properly (testing fluid). The testing fluid was incubated at 37^{O}C for 15-18 hours (first enrichment); 20 μl incubated fluid was added into 10 ml of 1X Alkaline peptone water (pH 8.6) and then incubated at 37^{O}C for 6 hours (second enrichment). The testing fluid was streaked on TCBS and Chrom-Vibrio agar, which were divided into 4 areas, and then incubated at 37℃ overnight.

b. Bacterial colonies were observed at the next day. Yellow or green bacterial colonies on TCBS agar plate and blue or purple bacterial colonies on Chrom-Vibrio agar were selected. Suspected bacterial colonies were then subcultured on TSA agar, incubated at 37℃, and then proceeded for bacterial identification by Vitek2 kit.

C. Data management and analysis:

Files of the amount of isolated bacteria and physical measurements were constructed by EXCEL. SPSS 14.0 statistic software was used for frequency distribution, chi square test and Wilcoxon rank sum test.

Results

A total of 204 samples were collected in this study and 476 strains of *Vibrio spp*. were isolated. In which, *Vibrio parahaemolyticus* was the most found bacterial strain (181 strains, 38%), and followed by *Vibrio alginolyticus* (154 strains, 32.4%), *Vibrio vulnificus* (94 strains, 19.7%), *Vibrio metschnikovii* (21 strains, 4.4%), *Vibrio fluvialis* (18 strains, 3.8%), *Aeromonas sobria* (7 strains, 1.5%) and other (1 strain, 0.2%). No *Vibrio cholerae* was isolated.

The geographic distribution and isolation rate of *Vibrio spp.* are shown in Table 1. The average isolation rate of *Vibrio parahaemolyticus*

Table 1. The number and average isolation rate of *Vibrio spp.* isolated from 17 harbors in Taiwan, 2009

Area	County/City	Harbor	Sample No.	*V. parahaemolyticus*	*V. alginolyticus*	*V. vulnificus*	*A. sobria*	*V. metschnikovii*	*V. fluvialis*
Taipei	Taipei	Tan Shui	12	11	11	6	0	1	0
	Keelung	Pa Tou Tzu	12	10	10	5	0	2	2
	Keelung	Cheng Pin	12	11	9	8	2	1	0
	Yilan	Nan Fang Ao	12	11	8	2	0	1	0
	Yilan	Wu Shih	12	10	10	7	1	1	2
		Subtotal	60	53	48	28	3	6	4
		Average isolation rate		88.3%	80.0%	46.7%	5.0%	10.0%	6.7%
North	Taoyuan	Chu Wei	12	9	10	5	0	2	2
	Hsinchu	Hsin Chu	12	10	10	5	0	0	1
		Subtotal	24	19	20	10	0	2	3
		Average isolation rate		79.2%	83.3%	41.7%	0.0%	8.3%	12.5%
Middle	Taichung	Wu Chi	12	12	10	5	0	0	0
	Changhua	Wang Kung	12	11	5	8	1	2	2
		Subtotal	24	23	15	13	1	2	2
		Average isolation rate		95.8%	62.5%	54.2%	4.2%	8.3%	8.3%
South	Yunlin	Po Tsai Liao	12	11	9	6	1	0	0
	Chiayi	Pu Tai	12	11	6	2	0	2	2
	Tainan	Gen. Ma Sha Kou	12	10	9	5	0	0	1
		Subtotal	36	32	24	13	1	2	3
		Average isolation rate		88.9%	66.7%	36.1%	2.8%	5.6%	8.3%
K.K.P.	Kaohsiung County	Hsin Ta	12	10	10	7	0	3	1
	Kaohsiung City	Chien Chen	12	12	8	6	0	4	3
	Pingtung	Fang Liao	12	12	9	8	1	2	1
	Pingtung	Yen Pu	12	10	8	7	1	0	1
		Subtotal	48	44	35	28	2	9	6
		Average isolation rate		91.7%	72.9%	58.3%	4.2%	18.8%	12.5%
East	Taitung	Cheng Kung	12	10	12	2	0	0	0
		Subtotal	12	10	12	2	0	0	0
		Average isolation rate		83.3%	100.0%	16.7%	0.0%	0.0%	0.0%
	p value			0.524	0.100	0.096	0.864	0.290	0.741
All areas		Total	204	181	154	94	7	21	18
		Average isolation rate		88.7%	75.5%	46.1%	3.4%	10.3%	8.8%

Note: Other *Vibrio spp*. were not listed in all areas was 88.7%, with the highest isolation rate (95.8%) noted in central Taiwan; average isolation rate of *Vibrio alginolyticus* was 75.5%, with the highest isolation rate (100%) recorded in eastern Taiwan; the average isolation rate of *Vibrio vulnificus* was 46.1%, with the highest isolation rate (58.3%) found in Kaohsiung and Pingtung area. Moreover, the average isolation rate of *Vibrio metschnikovii*, *Vibrio fluvialis* and *Aeromonas sobria* was low (3.4%-10.3%). There was no significant difference between the isolation of *Vibrio* spp. and geographic distribution by chi square test.

On isolation rate and seasonal distribution (Table 2), the highest average isolation rate for *Vibrio parahaemolyticus* was recorded in the fourth season (100%), while *Vibrio alginolyticus* is noted in the second season (88.2%), *Vibrio vulnificus* in the third season (76.5%), *Vibrio metschnikovii* in the fourth season (23.5%), *Vibrio fluvialis* in the third season (15.7%), and *Aeromonas sobria* in the first and third season (5.9%). Using chi square test,statistical significance of isolation of *Vibrio* spp. in different seasons was noted in *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio metschnikovii*, but not found in other *Vibrio spp.*

In physical measurements, water temperature in all areas was between 19.0 ℃～32.0℃, average 26.1 ℃; pH value in all areas was between 7.1～8.7, average 8.1; salinity in all areas was between 22～37, average 33. In different seasons, average water temperature was higher in the third season (30.4℃) than in other 3 seasons. No significant seasonal difference was recorded in average pH value and salinity. In 204 collected samples, *Vibrio parahaemolyticus* was isolated in 181 samples; *Vibrio alginolyticus*,154; *Vibrio vulnificus*,94; *Aeromonas sobria*,7; *Vibrio metschnikovii*,21, and *Vibrio fluvialis* ,18. By Wilcoxon rank sum test, statistical significance between isolation of *Vibrio* spp. and water temperature/salinity was noted in *Vibrio vulnificus*, while this was not found in other *Vibrio spp.* (Table 3).

Table 2. Number of samples isolated *Vibrio spp.* and isolation rate in 4 seasons in Taiwan area, 2009

Vibrio spp.	Sample No.	*V. parahaemolyticus*		*V. alginolyticus*		*V. vulnificus*		*A. sobria*		*V. metschnikovii*		*V. fluvialis*	
		Isolated No.	Isolation rate, %	Isolated No.	Isolation rate, %	Isolated No.	Isolation rate, %	Isolated No.	Isolation rate, %	Isolated No.	Isolation rate, %	Isolated No.	Isolation rate, %
1st season	51	39	76.5	39	76.5	14	27.5	3	5.9	1	2.0	2	3.9
2nd season	51	41	80.4	45	88.2	14	27.5	0	0.0	3	5.9	3	5.9
3rd season	51	50	98.0	35	68.6	39	76.5	3	5.9	5	9.8	8	15.7
4th season	51	51	100.0	35	68.6	27	52.9	1	2.0	12	23.5	5	9.8
p value		<0.001		0.069		<0.001		0.262		0.002		0.163	

Table 3. The correlation between isolation of *Vibrio* spp. and water temperature, pH value and salinity.

		V.parahaemolyticus		*V. alginolyticus*		*V. vulnificus*		*A. sobria*		*V. metschnikovii*		*V. fluvialis*	
	isolation	average	P value	average	P value	average	p value	average	p value	average	P value	average	P value
Temperature (℃)	positive	26.3	0.076	26.2	0.801	27.2	<0.001	26.4	0.873	26.3	0.797	27.3	0.158
	negative	24.8		26.0		25.2		26.1		26.1		26.0	
pH value	positive	8.1	0.076	8.2	0.054	8.1	0.652	8.2	0.688	8.1	0.244	8.1	0.258
	negative	8.2		8.1		8.1		8.1		8.1		8.1	
Salinity (‰)	positive	32.7	0.288	32.7	0.916	32.2	0.006	32.4	0.751	32.7	0.888	33.1	0.545
	negative	33.3		32.8		33.2		32.8		32.8		32.7	

Discussion

Traditional bacterial culture and isolation was used in our research and the result may be negative while the amount of pathogenic bacteria is low. Furthermore, pathogenic bacteria may become viable but non-culturable (VBNC) which could impair bacterial culture result.

As in previous investigation conducted by National Quarantine Service from 1991 to 1998, our study also selected harbors in northern, central, southern and eastern Taiwan for sea water sampling but different results were found. The isolation rate of *Vibrio parahaemolyticus* and *Vibrio vulnificus* was higher than previous research and no *Vibrio cholerae* was isolated while it was not low in previous study. The different results may be due to different sampling sites and/or VBNC [2, 7]. Geographically, *Vibrio parahaemolyticus* had the highest isolation rate at Wu Chi, Chien Chen and Fang Liao fishery harbor (100%), as well as *Vibrio alginolyticus* at Cheng Kung fishery harbor (100%). Isolation rate of *Vibrio vulnificus* at Cheng Pin, Wang Kung and Fang Liao fishery harbor was 66.7%. The highest isolation rate of *Vibrio metschnikovii*, *Vibrio fluvialis* and *Aeromonas sobria* was recorded at Chien Chen, Chien Chen and Cheng Pin fishery harbor respectively. In previous study conducted by National Quarantine Service, the highest isolation rate of *Vibrio parahaemolyticus* was found at Su Ao fishery harbor, while all other *Vibrio spp.* (*Vibrio alginolyticus, Vibrio vulnificus* , *Vibrio metschnikovii*, *Vibrio fluvialis* and *Aeromonas sobria*) were noted mainly at Keelung harbor.

According to other research, in the pelagic environment, 60% of total variance in culturable Vibrio data was explained by sea surface temperature (40%), salinity (13%) and organic matter concentration (7%). In the benthic environment, sea surface temperature was the only factor that significantly affected culturable Vibrio occurrence although it explained only 25% of total variance [8]. It was proved that temperature affected the isolation of pathogenic *Vibrio spp.* [6] and also had great effect in gene variety in recent studies [9]. The amount of *Vibrio spp.* varied with season changing in temperate zone, while it was not obvious in tropical zone [2]. Gene variety was also related to seasonal changing [9]. In our investigation, statistical difference was noted between isolation and seasonal changing in *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio metschnikovii.* For example, isolation rate of *Vibrio parahaemolyticus* in the fourth season (100%), *Vibrio vulnificus* in the third season and *Vibrio metschnikovii* in the fourth season (23.5%) were higher than other seasons. However, there was no significant seasonal changing in *Vibrio spp.* isolation in the previous study conducted by National Quarantine Service [5]. Furthermore, no statistical difference was recorded in *Vibrio spp.* isolation in different areas. Statistical significance was also found between *Vibrio vulnificus* isolation and water temperature as well as salinity. This result was similar to one previous study about *Vibrio vulnificus* distribution in southwestern cost of Taiwan in 2005 (the density of *V. vulnificus* appered to be controlled more by temperature than by salinity.) [10].

The average isolation rate of *Vibrio parahaemolyticus* was the highest, above

80% (79.2-95.8%) in all areas, which was also higher than another study about this pathogen in south-western Taiwan water in 2006 (40.7%) isolation rate in sea water samples and 51% isolation rate in organic samples) [11]. Another research indicated that *Vibrio parahaemolyticus* was most likely to be isolated from bottom mud and mollusk while water temperature higher than 15 ^{O} C and salinity lower than 30 [9]. *Vibrio parahaemolyticus* is an important pathogen for food poisoning. According to Taiwan FDA database from 1981-2008, 67.5% of food poisoning cases were caused by this pathogen (the most common pathogen causing food poisoning in history) [12]. Clinical sign was mainly diarrhea (mild to moderate) and usually self-limited. Primary septicemia was occasionally seen in few cases.

The average isolation rate of *Vibrio alginolyticus* was the second high and could be over 60% in each area (62.5-100%). In previous study conducted by National Quarantine Service, the bacteria had the highest isolation rate indicating wide distribution in Taiwan waters. These bacteria prefer water temperature between 17^{O} -35^{O}C and salinity between 5-25, and it is pathogenic to humans [13]. It is also pathogenic to many aquatic products, including sea fish (black porgy, yellow-finned black porgy, large yellow croaker, grouper), abalone, shrimp (tiger shrimp, kuruma prawn, white shrimp, fleshy prawn) and shellfish (clam, ormer), and may cause huge lost of aquaculture business [14]. Clinical signs of human infection are gastroenteritis or cutaneous/soft tissue infection (otitis). Respiratory infection or bacteremia is rarely seen. The first case of such clinical symptoms was reported in Taiwan in 2002, who was a 74 years old female breast cancer patient. She was infected by *Vibrio alginolyticus* due to eating raw sea food and developed pleural empyema and bacteremia [15].

The isolation rate of *Vibrio vulnificus* was the third high in our investigation. The average rate in each area was over 15% (16.7-58.3%), especially higher in southern area (58.3%). This result was similar to one research conducted in 2005 evaluating *Vibrio spp.* distribution in southwestern cost of Taiwan. This pathogen grows better in water temperature higher than 20^{O}C and salinity between 7-16 [3]. It is rarely seen when temperature under 17^{O}C [16]. In the United States, 95% of mortality caused by sea food was related to this pathogen and septicemia was the main cause of death. Patients with chronic hepatic diseases had about 80 times of risk in infection by ingesting raw oyster and had about 200 times of risk of death higher than other people [16]. In addition to chronic hepatic diseases, patients with hemochromatosis, AIDS, malignant tumors, immune deficiency and achlorhydria also had high risk of infection. The infection is usually due to accidental trauma and progresses very fast to septicemia. This may cause high mortality and thus critical medical care must be taken. Delayed treatment increases mortality and prevention is more important than medical treatment. In our result the highest isolation rate of this pathogen was recorded in southern Taiwan and in August. This result was similar to a 2004 study which analyzed the cases occurred from 1985-2000 and revealed that 90% of cases were found in southern Taiwan between April and October, with a high peak from June to August (summer season) [17].

One research in USA indicated that significant seasonal change of pathogenic *Vibrio spp.* isolation rate was noted. Over 90% cases occurred from April to October and probably due to shellfish/sea food consumption, oceanic entertainment and seasonal change of *Vibrio spp.* in the Gulf of Mexico [18]. *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* are believed to be the main pathogens for seafood-borne diseases. Consuming raw or unwell cooked sea food obtained from contaminated waters may increase risk of health. Coastal entertainment areas associated with recreational fisheries were established in recent years and sea food tasting or related activities are usually arranged for public. Authorities of public health and fishery should inform public, especially those who have hepatic diseases or chronic subclinical diseases, be aware of accidental injury when handling sea food, contact sea water or consuming unwell cooked food. Restaurants or retailers selling raw or unwell cooked food should be encouraged to establish signs or warning for these high risk people, and sea food should be rinsed thoroughly by fresh water and disinfected by low temperature or high pressure [16]. Furthermore, medical doctors should also remind these high risk patients about the possibility of risk when consuming raw sea food.

Vibrio spp. outbreak may also be caused by natural disasters. In August 2005, super hurricane Katrina occurred which caused severe flooding in south-eastern USA and then followed by community infection event of *Vibrio spp.* From August 29 to September 1, twelve *Vibrio spp.* infections were reported to Mississippi Health Department by medical facilities at Gulf of Mexico areas, in which 8 cases of *Vibrio vulnificus*, 2 cases of *Vibrio parahaemolyticus*, 1 case of *Vibrio fluvialis* and one other. The average age of these patients was 76 years old (range: 60 to 83 years old); 75% of patients had chronic diseases; 9 patients had obvious wound; and 4 patients died on arriving hospital [18]. This information indicated that sea water exposure caused by natural disaster may also induce severe infection of high risk patients. During our investigation, Typhoon Morakot caused severe flood disaster in southern Taiwan. Community infection event of Leptospirosis (106 cases) and increased Melioidosis cases (25 cases) were reported. However, no obvious increase of *Vibrio spp.* infection event was noted. Typhoon or heavy rain occurs every year in Taiwan area. Although no sudden increase of *Vibrio spp.* infection event after flooding, high risk people who reside at coastal areas or low-lying areas should be informed the risk of infection. Medical facilities and staffs should also be aware of this event and should be careful in diagnosis and treatment for this case.

Recommendations

1. Authorities including public health, environmental protection and fishery should monitor the distribution of pathogenic *Vibrio spp.* regularly and the public should be informed on the related information.

2. Environmental and clinical bacterial database, gene mappings and information of drug resistance should be established by laboratory examination and compared with bacterial strains from other countries in order to provide reference and recommendation for disease prevention and food hygiene policies.

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