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＝Original＝

Isolation of *Vibrio vulnificus* from Commercially Available Saltwater Fishes, and Isolates Serotyping and Antibiotics Resistance

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To clarify the infection routes and sources as a part of basic studies on *Vibrio vulnificus* (*V. vulnificus*) infection, we evaluated the contamination status of commercially available seawater fish by this bacterium, and performed serotyping and susceptibility tests using various drugs in this study. The following results were obtained:

1. This bacterium was isolated from 57 (5.4%) of 1,049 fresh fish samples.
2. The isolation status according to region, this bacteria was isolated from 1 (0.6%) of 157 samples from Tottori, 21 (13.6%) of 154 samples from Tokushima, 26 (28.9%) of 90 samples from Ehime, and 9 (2.0%) of 443 samples from Kanagawa indicating that Tokushima and Ehime showed high isolation rates, identifying a regional difference.
3. According to fish species, this bacterium was isolated from 53 (8.6%) of 613 horse mackerel samples, 4 (2.1%) of 191 sardine samples, and 1 (3.7%) of 27 barracuda samples.
4. According to examination sites, this bacterium was isolated from the visceral organs in 14 samples (1.3%), the gills in 18 (1.7%), and the body surface in 26 (2.5%).
5. As a result of serotyping, 32 (55.2%) of the 58 examined strains were differentiated into 9 serotypes; serotype O22 was the most frequently observed (19.0%), followed by O4 (10.3%).
6. The results of drug susceptibility tests were compared in terms of MIC_{90}. All strains were susceptible to GM, EM, TC, DOXY, MINO, CP, NA, and CPFX. However, some strains were resistant to ABPC, PIPC, CER, CET, CPZ, CTX, CMZ, LMOX, MEPM, KM, AMK, or LCM.

Key words: *Vibrio vulnificus*, drug susceptibility, sero type, saltwater fish

Introduction

Vibrio vulnificus is the first report that Roland[14] isolated from the skin exudate of the patient leg in 1970 on the human infectious disease by this bacteria. A characteristic of this bacteria cause the intestine outside infection unlike other *Vibrio*, does not infect healthy human and human with the basal disease in livers, etc. and the human who the immune strength lowers are infected, and the serious infectious disease has been caused[2].

As a part of basic studies to clarify associations with various infections due to *V. vulnificus* and infection sources, we previously performed a distribution survey of this bacterium in saltwater, sea mud, and oysters in the natural environment, and showed its high isolation rate from summer to autumn[12]. In addition, we performed drug susceptibility tests of natural environment-derived strains and human clinical isolates, and observed a high incidence of resistant strains in clinically isolated strains and marked incidences of serotypes O4 and O7 in strains with both derivations[13]. However, there have been no surveys on the contamination of commercially available saltwater fish as an estimated source of *V. vulnificus* infection.

Therefore, we evaluated the status of contamination by this bacterium and performed serotyping and drug susceptibility tests using samples of mainly horse mackerel and sardines as popular kinds of fish often eaten raw.

Materials and Methods

1. Materials

The materials were 1,049 samples of fresh fish purchased from 9 general fishmongers (Nagasaki, Tottori, Tokushima, Ehime, Niigata, Kanagawa, Chiba, Miyagi, and Hokkaido Prefectures; 1 each) between May 2003 and September 2004 (613 horse mackerel, 191 sardines, 55 sandfish, 50 saury, 48 Japanese bluefish, 30 plaice, 27 barracuda, 24 amberjack, 6 flying fish, 3 sand borers, and 2 mackerel).

2. Isolation and identification

Each fish was divided into the visceral organs, gills, and body surface and processed by the following methods.

Visceral organs: The visceral organs (2–4 g) were aseptically removed from the fish body and cultured with about 10 volumes (20–40 m*l*) of alkaline peptone water supplemented with 0.08% cellobiose and polymyxin B (APWPC, Merck) at 37℃ for 18 hours[5].

Gills: The gills were removed and cultured in 20 m*l* APWPC at 37℃ for 18 hours.

Body surface: The body surface was wiped with sterile cotton swabs, which were placed in 5 m*l* APWPC, stirred thoroughly, and cultured for enrichment at 37℃ for 18 hours.

After the enrichment culture of each part of fish, 1 loop of culture solution was obtained, smeared onto cellobiose agar supplemented with colistin (CCA), and cultured at 37℃ for 18 hours[6]. Yellow colonies that degraded cellobiose were suspected to be *V. vulnificus*, picked, and grown by pure culture.

The bacterium was identified using Enteogram (Wako Pure Chemicals, Co., Ltd.) by the method previously described[12].

3. Serotyping

Serotyping was performed according to our previously reported method[13, 16] using a total of 21 serotypes from O1 to O23 (lacking O17 and O18).

4. Drug susceptibility tests

Drug susceptibility tests were performed by the agar plate dilution method according to the standard method of the Japanese Society of Chemotherapy[7]. A total of 20 drugs were used: 9 β-lactam drugs, 2 quinolone drugs, 1 macrolide drug, 1 chloramphenicol drug, 3 tetracycline drugs, 3 aminoglycoside drugs, and 1 lincomycin drug. (Table 1)

Results

1. Isolation status of *V. vulnificus* from fresh fish samples

V. vulnificus was isolated from 57 (5.4%) of

Table 1. Drug list for determination of MIC

Drug
β-Lactam antimicrobial
Penicillin antimicrobial
Ampicillin (ABPC: Sigma), Piperacillin (PIPC: Sigma)
Cephalosporins antimicrobial
Cefaloridine (CER: Sigma), Cefalotin (CET: Sigma), Cefoperazone (CPZ: Sigma), Cefotaxime (CTX: Chugai), Cefmetazole (CMZ: Sankyo), Latamoxef (LMOX: Shionogi)
Penem antimicrobial
Meropenem (MEPM: Sumitomo)
Quinolone antimicrobial
Nalidixic acid (NA: Sigma), Ciprofloxacin (CPFX: Bayer)
Macrolide antimicrobial
Erythromycin (EM: Sigma)
Chloramphenicol antimicrobial
Chloramphenicol (CP: Sigma)
Tetracyclines antimicrobial
Tetracycline (TC: Sigma), Doxycycline (DOXY: Sigma), Minocycline (MINO: Sigma)
Aminoglycoside antimicrobial
Kanamycin (KM: Sigma), Amikacin (AMK: Sigma), Gentamicin (GM: Schering-Plough)
Lincomycin antimicrobial
Lincomycin (LCM: Sigma)

Table 2. Seasonal frequency of *V. vulnificus*

Area	Season				Total
	Spring (March–May)	Summer (June–August)	Autumn (Sep.–Nov.)	Winter (Dec.–Feb.)	
West Japan					
Nagasaki		0/ 40			0/ 40
Tottori	0/12*[1]	1/145 (0.7%)			1/ 157 (0.6%)
Tokushima		21/154*[2] (13.6%)			21/ 154 (13.6%)
Ehime		26/ 90 (28.9%)			26/ 90 (28.9%)
Sub total	0/12	48/429 (11.2%)			48/ 441 (10.9%)
East Japan					
Niigata		0/ 43			0/ 43
Kanagawa	0/62	3/178 (1.7%)	6/150 (4.0%)	0/53	9/ 443 (2.0%)
Chiba		0/ 72			0/ 72
Miyagi			0/ 25		0/ 25
Hokkaido		0/ 25			0/ 25
Sub total	0/62	3/318 (0.9%)	6/175 (3.4%)	0/53	9/ 608 (1.5%)
Total	0/74	51/747 (6.8%)	6/175 (3.4%)	0/53	57/1049 (5.4%)

*[1] No. of positive/No. of examined
*[2] Overlaps

the 1,049 fresh fish samples. In one (Tokushima, horse mackerel) of the 57 samples, this bacterium was isolated from 2 examination sites (visceral organs and gills). The isolation status according to region is shown in Table 2. This bacterium was isolated from 1 (0.6%) of 157 samples from Tottori, 21 (13.6%) of 154 samples from Tokushima, 26 (28.9%) of 90 samples from Ehime, and 9 (2.0%) of 443 samples from Kanagawa, but nor from Nagasaki, Niigata, Chiba, Miyagi, and Hokkaido.

Regarding isolation rates of *V. vulnificus* according to season this bacterium was isolated from 51 (6.8%) of 747 samples in summer, and 6 (3.4%) of 175 samples in autumn. However, in spring and winter, no strain was isolated. The isolation rates of *V. vulnificus* according to fish species are shown in Table 3. This bacterium was isolated from 52 (8.5%) of 613 horse mackerel. By area, the bacteria were most frequently isolated in samples from Ehime 26 of 90 samples (28.9%), followed by 21/149 (14.1%) from Tokushima and 5/351 (1.4%) from Kanagawa, but in none of the samples from Nagasaki or Tottori. This bacterium was isolated from 4 (2.1%) of 191 sardines. By area, the bacteria were most frequently isolated in samples from Kanagawa: 3 of 63 samples (4.8%)and followed by 1/104 (1.0%) from Tottori, but in none of the samples from Nagasaki or Tokushima. Regarding barracuda, bacteria were isolated from 1 of 27 samples (3.7%). By region, they were isolated in 1 of 26 samples (3.8%) from Kanagawa, but not in any sample from Tokushima.

The isolation rates of *V. vulnificus* according to examination site are shown in Table 3. This bacterium was isolated from 14 (1.3%) of 1049 visceral organs. By area, the bacteria were most frequently isolated in samples from Tokushima: 7 of 154 samples (4.5%), followed by 5/443 (1.1%) from Kanagawa, 1/90 (1.1%) from Ehime and 1/157 (0.6%) from Tottori, but in none of the samples from Nagasaki, Niigata, Chiba, Miyagi or Hokkaido. This bacterium was isolated from 18 (1.7%) of 1049 gills. By area, the bacteria were most frequently isolated in samples from Tokushima: 15 of 154 samples (9.7%), followed by 3/443 (0.7%) from Kanagawa, but in none of the samples from Nagasaki, Ehime, Tottori, Niigata, Chiba, Miyagi or Hokkaido. Regarding the body surface, bacteria were isolated from 26 of 1049 samples (2.5%). By region, the bacteria were most frequently isolated in samples from Ehime: 25 of 90 samples (27.8%), followed by 1/443 (0.2%) from Kanagawa, but in none of the samples from Nagasaki, Tokushima, Tottori, Niigata, Chiba, Miyagi or Hokkaido.

2. Status of isolate serotyping

The status of serotyping of the 58 strains isolated from fresh fish samples is shown in Table 4. Of the 58 strains, 32 (55.2%) were differentiated into 9 serotypes. Of the 53 strains isolated from horse mackerel, 31 (58.5%)

Table 3. Class of saltwater fish and isolation position frequency of *V. vulnificus*

	Nagasaki	Tottori	Tokushima	Ehime	Niigata	Kanagawa	Chiba	Miyagi	Hokkaido	Total
Species of fish										
Horse mackerel (*Trachurus japonicus*)	0/20*	0/ 3	21/149 (14.1%)	26/90 (28.9%)		5/351 (1.4%)				52/ 613 (8.5%)
Sardine (*Sardinops melanostictus*)	0/20	1/104 (1.0%)	0/ 4			3/ 63 (4.8%)				4/ 191 (2.1%)
Sandfish (*Arctoscopus japonicus*)		0/ 12			0/43					0/ 55
Saury (*Cololabis saira*)								0/25	0/25	0/ 50
Japanese bluefish (*Scombrops* spp.)							0/48			0/ 48
Plaice (*Pleuronectes* spp.)		0/ 30								0/ 30
Barracuda (*Sphyraena* spp.)			0/ 1			1/ 26 (3.8%)				1/ 27 (3.7%)
Amberjack (*Seriola dumerili*)							0/24			0/ 24
Flying fish (*Cypselurus* spp.)		0/ 6								0/ 6
Sand borers (*Sillago* spp.)						0/ 3				0/ 3
Mackerel (*Scomber* spp.)		0/ 2								0/ 2
Total	0/40	1/157 (0.6%)	21/154 (13.6%)	26/90 (28.9%)	0/43	9/443 (2.0%)	0/72	0/25	0/25	57/1049 (5.4%)
Isolation position										
Visceral organs		1/157 (0.6%)	7/154 (4.5%)	1/90 (1.1%)		5/443 (1.1%)				14/1049 (1.3%)
Gills			15/154 (9.7%)			3/443 (0.7%)				18/1049 (1.7%)
Body surface				25/90 (27.8%)		1/443 (0.2%)				26/1049 (2.5%)

* No. of positive/No. of examined

Table 4. Serotype of *V. vulnificus* in class of area, species and isolate position

	No. of positive	O serotype										
		4	6	7	8	14	16	19	22	23	UT*	Self-aggregation
Total	58	6 (10.3%)	4 (6.9%)	1 (1.7%)	3 (5.2%)	3 (5.2%)	2 (3.4%)	1 (1.7%)	11 (19.0%)	1 (1.7%)	21 (36.2%)	5 (8.6%)
Area												
Tottori	1 (0.6%)	1 (100%)										
Tokushima	22 (14.3%)	1 (4.5%)	4 (18.2%)	1 (4.5%)	1 (4.5%)	3 (13.6%)	2 (9.1%)		1 (4.5%)	1 (4.5%)	5 (22.7%)	3 (13.6%)
Ehime	26 (28.9%)	3 (11.5%)			1 (3.8%)				10 (38.5%)		11 (42.3%)	1 (3.8%)
Kanagawa	9 (2.0%)	1 (11.1%)			1 (11.1%)			1 (11.1%)			5 (55.6%)	1 (11.1%)
Species of fish												
Horse mackerel (*Trachurus japonicus*)	53 (8.6%)	5 (9.4%)	4 (7.5%)	1 (1.9%)	3 (5.7%)	3 (5.7%)	2 (3.8%)	1 (1.9%)	11 (20.8%)	1 (1.9%)	18 (34.0%)	4 (7.5%)
Sardine (*Sardinops melanostictus*)	4 (2.1%)	1 (25.0%)									2 (50.0%)	1 (25.0%)
Barracuda (*Sphyraena* spp.)	1 (3.7%)										1 (100%)	
Isolate position												
Visceral organs	14 (1.3%)	1 (7.1%)	2 (14.3%)		1 (7.1%)	1 (7.1%)		1 (7.1%)			6 (42.9%)	2 (14.3%)
Gills	18 (1.7%)	2 (11.1%)	2 (11.1%)	1 (5.6%)		2 (11.1%)	2 (11.1%)		1 (5.6%)	1 (5.6%)	5 (27.8%)	2 (11.1%)
Body surface	26 (2.5%)	3 (11.5%)			2 (7.7%)				10 (38.5%)		10 (38.5%)	1 (3.8%)

* UT: untypeable

were differentiated into 9 serotypes; serotype O22 was observed in 11 strains (20.8%), O4 in 5 (9.4%), O6 in 4 (7.5%), and O8 and O14 in 3 each (5.7%), but 18 strains (34.0%) were untypeable, and 4 (7.5%) showed autoagglutination. Of the 4 strains derived from sardines, 1 (25.0%) was serotyped as O4, but 2 were untypeable (50.0%), and the other (25%) showed autoagglutination. The one strain derived from a barracuda was untypeable. As a reslt of the serotyping status according to examination site, of the 14 strains from the visceral organs, 6 (42.9%) were differentiated into 5 serotypes: serotype O6 was observed in 2 strains (14.3%), O4, O8, O14, and O19 were observed in 1 each (7.1%), 6 strains (42.9%) were untypeable, and 2 showed autoagglutination (14.3%). Of the 18 strains from the gills, 11 (51.1%) were differentiated into 7 serotypes: serotypes O4, O6, O14, and O16 were observed in 2 each (11.1%), and O7, O22, and O23 in 1 each (5.6%), 5 strains (27.8%) were untypeable, and 2 (11.1%) showed autoagglutination. Of the 26 strains from the body surface, 15 (57.7%) were differentiated into 3 serotypes: serotype O22 was observed in 10 strains (38.5%), O4 in 3 (11.5%), and O8 in 1 (7.7%), 10 strains (38.5%) were untypeable, and one (3.8%) showed autoagglutination. The serotyping status according to region is shown in Table 4. The one strain derived from Tottori was differentiated into O4. Of the 22 strains from Tokushima, 14 (63.6%) were differentiated into 8 serotypes: serotype O6 was observed in 4 strains (18.2%), O14 in 3 (18.2%), O16 in 2 (9.1%) and O4, O7, O8, O22, and O23 in 1 each (4.5%), 5 strains (22.7%) were untypeable, and 3 (13.6%) showed autoagglutination. Of the 26 strains from Ehime, 14 (53.8%) were differentiated into 3 serotypes: serotype O22 was observed in 10 strains (38.5%), O4 in 3 (11.5%), and O8 in 1 (3.8%), 11 strains (42.3%) were untypeable, and one (3.9%) showed autoagglutination. Of the 9 strains from Kanagawa, 3 (53.8%) were differentiated into 3 serotypes: serotype O4, O8, and O19 were observed in 1 each (11.1%), 5 strains (55.6%) were untypeable, and one (11.1%) showed autoagglutination.

3. MIC distribution status of various drugs

The MIC distribution and MIC_{90} of various drugs for the 58 isolated strains are shown in Table 5. Among penicillin antimicrobials, ABPC showed a monophasic MIC distribution (peak, 12.5 μg/ml) while PIPC showed a biphasic distribution (peaks, 0.2 and 25 μg/ml). Among cephem antimicrobials, the MIC distribution was monophasic for CMZ (peak, 25 μg/ml), biphasic for CER (peaks, 12.5 and 100< μg/ml), CET (peaks, 12.5 and 100 μg/ml), CPZ (peaks, 0.2 and 12.5 μg/ml), LMOX (25 and 100< μg/ml), and MEPM (peaks, 0.05 and 50 μg/ml), and triphasic for CTX (peaks, 0.05, 3.13, and 50 μg/ml). Concerning aminoglycoside antimicrobials, a monophasic MIC distribution was observed for KM (peak, 25 μg/ml), GM (peak, 6.25 μg/ml), and AMK (peak, 25 μg/ml). As a lincomycin antimicrobial, LCM showed a monophasic MIC distribution (peak, 100< μg/ml). As a macrolide antimicrobial, EM exhibited a monophasic MIC distribution (peak, 3.13 μg/ml). Among tetracycline antimicrobials, the MIC distribution was biphasic for TC (peaks, 0.2 and 6.25 μg/ml) and DOXY (peaks, 0.2 and 1.56 μg/ml) and monophasic for MINO (a peak, 0.05 μg/ml). As a chloramphenicol antimicrobial, CP showed a monophasic MIC distribution (peak, 0.78 μg/ml). As quinolone antimicrobials, the MIC distribution was monophasic for NA (peak, 0.39 μg/ml) but biphasic for CPFX (peaks, 0.025 and 0.2 μg/ml).

Evaluation of MIC_{90} showed that all strains

Table 5. Susceptibility distribution of saltwater fish isolates

Drug	MIC* range (μg/ml)	Peak points (μg/ml)	MIC_{90} (μg/ml)
ABPC	6.25 ～100<	12.5	50
PIPC	0.025～100	0.2, 25	50
CER	6.25 ～100<	12.5, 100<	100
CET	6.25 ～100<	12.5, 100	12.5
CPZ	0.05 ～100	0.2, 12.5	25
CTX	0.025～ 50	0.05, 3.13, 50	12.5
CMZ	3.13 ～100<	25	50
LMOX	0.1 ～100<	25, 100<	25
MEPM	0.025～100	0.05, 50	25
KM	6.25 ～ 50	25	50
GM	1.56 ～ 12.5	6.25	6.25
AMK	3.13 ～ 50	25	25
LCM	50 ～100<	100<	100<
EM	0.2 ～ 12.5	3.13	6.25
TC	0.1 ～ 6.25	0.2, 6.25	0.39
DOXY	0.1 ～ 1.56	0.2, 1.56	0.2
MINO	0.05 ～ 0.2	0.05	0.1
CP	0.39 ～ 1.56	0.78	0.78
NA	0.2 ～ 1.56	0.39	1.56
CPFX	0.025～ 0.78	0.025, 0.2	0.05

* MIC: minimum inhibitory concentration

were susceptible to GM, EM, TC, DOXY, MINO, CP, NA, and CPFX, but some strains were resistant to ABPC, PIPC, CER, CET, CPZ, CTX, CMZ, LMOX, MEPM, KM, AMK, or LCM.

Discussion

As a part of basic studies on *V. vulnificus* infection, we carried out an epidemiological study by evaluating the status of *V. vulnificus* contamination of commercially available saltwater fish that are often eaten raw and also by performing serotyping and drug susceptibility tests of isolated strains. Cerda-Cuellar *et al.*[1] reported that, generally, the isolation rate varies depending on the medium used for direct isolation, whether enrichment culture is performed before isolation culture, and among combinations of these. We used CCA after enrichment culture with APWPC, and isolated bacteria from 5.4% of commercial marine fish. Tanaka *et al.*[18] in Tottori Prefecture and Fukushima *et al.*[3] in Shimane Prefecture also attempted to isolate bacteria by enrichment culture with alkaline peptone water (APW) followed by isolation culture with CHROMagar Vibrio medium (CHV), an enzyme substrate medium, and obtained isolation rates of 8.6% and 10.5%, respectively, showing rates higher than that obtained by us. In a study performed by Ohata *et al.*[10] in Shizuoka Prefecture, bacteria were isolated using CHV and mCPC medium after enrichment culture with APW, and the isolation rate was 5.0%, similar to our finding, but Saito *et al.*[15] in Miyagi Prefecture obtained no isolate although they used the same enrichment and isolation media (APW and mCPC), respectively.

According to season, in Kanagawa where fish can be bought all year, the isolation rate was 1.7% in summer and 4.0% in autumn, but no strain was isolated in spring and winter. Tanaka *et al.*[18] reported rates of 12.7% in summer and 11.4% in autumn. However, no isolation occurred in any samples in spring and winter. We performed isolation in the same season as Tanaka *et al.*, but the isloation rate was higer in their study. Saito *et al.* performed a survey of commercially available fish in Miyagi, but did not isolate this bacterium from any sample throughout the year. Yano *et al.*[19] performed a survey of fish purchased from a fish market in China in summer and autumn, but did not isolate this bacterium from any sample. In this study, the isolation rate according to examination site was 1.3% in the visceral organs, 1.7% in the gills, and 2.5% on the body surface. Sunahara *et al.*[17] investigated gizzard shads and flatfish, and isolated bacteria from 21.4% and 23.2% of visceral organ and skin samples, respectively. Compared to these findings, the isolation obtained by us was only about 1/9–1/16. Differences in the fish species, water temperature at the production site, and distribution conditions till purchase by consumers may have affected the isolation rate. On comparison by fish species, the isolation rates from horse mackerel landed at fishing ports along the Seto Inland Sea in Ehime and Tokushima were high: 28.9% and 14.1%, respectively, but those in Kanagawa, Nagasaki, and Tottori were low. On comparison of the detection rate, although the fish species was different, Sunahara *et al.*[17] in Kagawa Prefecture reported a value of 22.3%, similar to our finding, suggesting that the sea salt concentration in the fisheries in the Seto Inland Sea is suitable for bacterial growth. The Tsushima Current may also be related to the low isolation rates from marine fish from Nagasaki and Tottori. Regarding factors that may affect the low isolation rate from marine fish from Kanagawa, since the survey was performed throughout the year, unlike those performed in other regions, seasons with characteristically low isolation rates may have been included.

Concerning the status of serotyping, 55.2% of the examined strains could be differentiated into 9 serotypes. Serotypes O22, O4, and O6 were frequently observed. The serotypes observed in this study included O6 and O4, which are also observed in human clinical isolates, which suggests the pathogenicity of these serotypes in humans. Though the relation between the pathogenicity to humans and the specific serotype is not always consistent, the possibility of a relation based on acrual disease-causing facter should be considered. The serotypes observed differed between regions. In Tottori, serotype O4 was observed, in Tokushima, there were 8 serotypes represented by O4, O6, O7, O8, O14, O16, O22, and O23, in Ehime, 3 serotypes (O4, O8, and O22) were

noted, and in Kanagawa, there were 3 serotypes represented by O4, O8, and O19. These results indicate regional differences in serotypes, and strains with a different serotype. Moreover, in horse mackerel from Ehime Prefecture, investigated at one time, isolates from the body surface were typed O22 in many fishes, suggesting the possibility that steps from capture to distribution were secondarily contaminated, but the site of fish capture was not. In contrast, in horse mackerel from Tokushima, bacteria were isolated from multiple regions at a high frequency, and various serotypes were identified, suggesting that the sea area was contaminated, rather than there being secondary contamination. Regarding a comparison of serotypes between the isolates from the investigated environmental sites and patients, Okada *et al.* reported serotype O3 in 38.5%, O4 in 23.1%, O14 in 15.4%, and O1 and O6 in 7.7% each, and 7.7% untypeable strains. In a study performed by Miyasaka *et al.*[8, 9] in Kumamoto Prefecture, O4A, O7, and O3/O6 were typed in 55.6%, 33.3%, and 11.1% of human clinical isolates, respectively, and O1 and O6 were each typed in 20.0%, O4 in 14.5%, O7 in 3.6%, and O3 and O5 in 1.8% each of isolates from waterfowl, but the remaining 38.3% could not be typed. In Shimane Prefecture, Fukushima *et al.*[4] determined serotypes of isolates from fish and shellfish, and found that O4 was most frequently typed (22.3%), followed by O1 in 17.0%, O3 in 16.0%, O8 in 6.4%, O6 in 4.3%, O5 and O12 in 3.2% each, and O9, O14 and O16 in 1.1% each, but the remaining isolates could not be typed, or could be typed non-specifically. On comparison with our findings, although no isolate was typed O1 or O3 in our study, the isolation of other types was similar.

Drug susceptibility tests of isolated strains were performed, and MIC_{90} was compared. All strains derived from commercially available fish were susceptible to GM, EM, TC, DOXY, MINO, CP, NA, and CPFX. However, some strains were resistant to ABPC, PIPC, CER, CPZ, CMZ, LMOX, MEPM, KM, or AMK. We previously evaluated human clinical isolates and environment-derived isolates and reported a high incidence of resistant strains in the former. Comparison between these results and those of the present study suggests that strains derived from fish in the natural environment have also become increasingly resistant.

Thus, *V. vulnificus* was also isolated from commercially available fish in summer and autumn, which confirmed contamination by this bacterium. Serotyping showed high incidences of O4 and O6, which are consistent with the serotypes in human clinical isolates, indicating the possibility that *V. vulnificus* may infect humans via consuming infected commercially available fish. Drug susceptibility tests suggested that strains derived from the natural environment have also become increasingly resistant.

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市販海産魚類からの *Vibrio vulnificus* の分離と分離菌株の血清型別および薬剤耐性

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Vibrio vulnificus 感染症に関する基礎的研究の一環として感染経路や感染源を解明するため，今回は市販海産魚類の本菌の汚染状況，血清型別および各種抗菌剤の薬剤感受性試験を行ったところ，以下の成績が得られた．

1. 新鮮魚1049例について本菌の分離を試みたところ，57例(5.4%)から本菌が分離された．
2. 産地別における分離状況は，鳥取県が157例中1例(0.6%)，徳島県が154例中21例(13.6%)，愛媛県が90例中26例(28.9%)，神奈川県が443例中9例(2.0%)からそれぞれ本菌が分離され，徳島県と愛媛県が高い分離率を示し，産地間の差異が認められた．
3. 魚種別では，アジ613例中53例(8.6%)，イワシ191例中4例(2.1%)およびカマス27例中1例(3.7%)からそれぞれ本菌が分離された．
4. 検査部位別では内蔵14例(1.3%)，エラ18例(1.7%)および体表26例(2.5%)からそれぞれ本菌が分離された．
5. 血清型別状況では，供試した58株中32株(55.2%)が9菌型に型別され，O22が19.0%と最も多く，次にO4が10.3%などであった．
6. 薬剤感受性試験結果をMIC_{90}値で比較すると，GM, EM, TC, DOXY, MINO, CP, NAおよびCPFXに対して全株が感受性を示したが，ABPC, PIPC, CER, CET, CPZ, CTX, CMZ, LMOX, MEPM, CTX, KMA, MKおよびLCMに対しては耐性株が認められた．