Water Quality and Isolation of *Vibrio cholerae* non-O1 from an Aquatic Environment

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Key words: *Vibrio cholerae* non-O1, *Environmental microbacteria*, *Water quality*

Abstract

According to reports hitherto on the isolation of *Vibrio cholerae* non-O1 from the environment, environmental conditions at the time of detection differ depending on whether isolation is from the sea or rivers.

We based this study on two rivers of differing water quality and studied the trend of a bacteria indicator to observe the conditions of existence of *V. cholerae* non-O1.

1) It was thought that *V. cholerae* non-O1 did not flow from the land or other areas of pollution but existed at those points in the river from which they were isolated.

2) The proportion of *V. cholerae* non-O1 isolated was higher for the river with a higher degree of water pollution and higher in areas of high pollution in both rivers.

3) In rivers with a low degree of pollution or having a small range in water quality, an increase in Cl^- and PO_4^{3-} ions, or an increase in the number of bacteria grown on nutrient agar medium at 37°C for 24 hours appears to increase the frequency of isolation of *V. cholerae* non-O1.

4) Approximately 13% of the samples of isolated *V. cholerae* non-O1 exhibited the cholera enterotoxin strain.

Introduction

It has been reported that *V. cholerae* non-O1 is frequently isolated after outbreaks of *V. cholerae* O1¹⁾. Furthermore it is considered that both *V. cholerae* O1 and non-O1 can exist for prolonged periods according to the environmental conditions.

It has also been reported that it is highly probable that *V. cholerae* non-O1 exists as an aquatic bacterial flora²⁾. That is, after contaminating a river, *V. cholerae* non-O1 is thought to be able to exist like bacterial flora in the aquatic environment, when it co-exists with other organisms. It is estimated that with infection of the human body by *V. cholerae* non-O1 there is also an included risk of infection and the danger of existence of *V. cholerae* O1 which is merely different in serotype from *V. cholerae* non-O1.

In general, water quality and the composition of living organisms in the river are used to observe a river's environmental conditions.

We examined the conditions for the isolation of *V. cholerae* non-O1 and the river's microbiological, physical and chemical constituents and investigated the relationship between the river's environmental factors and the conditions of existence for *V. cholerae* non-O1.

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Materials and Methods

1. Location and duration of survey: Figure 1 shows the four locations on the Sagami River and five locations on the Sakai River that were selected. Sagami-hara city is situated between the rivers. The survey was carried out during six months from July to December, 1985, at each of the locations and the river water sampled five times at approximately monthly intervals. At each station samples of river water were taken from two places; one being the center of running water and the other being from still water at the edge of the river.

Near location 1 on the Sagami R., water is discharged from the Tsukui Dam. Location 2 is upstream from a filtration plant's intake waterway flood gate. There is a camping ground on the river beach between locations 1 and 2. At location 3 the river is wide and fishing boats come and go. Upstream from this location very polluted the Hato R. flows in. At location 4 there is a filtration plant and a barge just upstream and, at high tide, the seawater flows back to this location.

The Sakai R. flows from the Lake Shiroyama reservoir but is joined with the inflow of domestic drainage directly below this and becomes very polluted. Location 5 is located on the river where the width is less than one meter and the water flow is low. At location 6 the lathering of soap suds is already prevalent. At location 7 the river flows through a city and is joined by much drainage. Around location 8 are many tall buildings and there are many suspended particles in the river. Location 9 is only 0.8 km from an estuary. We believe that all environmental features are covered by the locations on the two rivers mentioned in the foregoing script. This survey covers approximately 30 km of each river.

2. Water quality examination methods (physical and chemical tests): Air temperature was measured with a mercury thermometer. Water temperature was measured with a Pettenkoffer's Water-thermometer. The degree of transparency was measured with a 30 cm transparency measuring device. The pH was measured by colorimetry. The amount of ammonia nitrogen (NH_4^+) was measured using Nessler method and phosphate ion (PO_4^{3-}) was measured using the ammonium molybdate method. Dissolved oxygen (DO) was measured by electrode method. The above analyses were performed immediately after taking the water samples. In addition, a 2000 ml portion of the sampled water was carried in polyethylene tank to our laboratory and examined. The sodium cation (Na^+) and the chlorine anion (Cl^-) were measured by using the selective electrode method and water hardness was determined by the EDTA method. Electrical conductivity (EC) was measured with an electrical conductivity meter and the salinity concentrations were measured using a refractometer with a minimum scale of 1‰.

3. Bacteriological analyses: The water samples were carried to our laboratory and analysed within 4

Fig. 1. Location of sampling stations in the Sagami River and the Sakai River.

1. Ogura-hashii; 2. Shouwa-hashii; 3. Sagami-ohashi; 4. Kamikawa-hashii; 5. Shiroyama-sakai-hashii; 6. Negisi-hashii; 7. Sakai-hashii; 8. Midori-hashii; 9. Arayashiki-hashii.

Table 1 The identification of *Vibrio cholerae* non-O1

Gram reaction	—	Oxidation-Fermentation (glucose)	F
Shape	Rod	Glucose (gas)	—
Spore	—	Lactose (acid)	±
Motility	+	Sucrose (acid)	+
Citrate utilization	+	Mannitol (acid)	—
Gelatin hydrolysis	+	Inositol (acid)	—
Urease	—	Arabinose (acid)	—
Indol	+	Maltose (acid)	+
H ₂ S	—	Salicin (acid)	—
Lysine decarboxylase	+	Growth in NaCl (% concentration)	
Arginine dihydrolase	—	0	+
Ornithine decarboxylase	+	3	+
Voges-Proskauer	±	7	—
Cytochrome-Oxidase	+	10	—
Nitrate reduction	+	0/129 sensitivity	+
Catalase	+	Anti-O1 serm	—
Oxidase	+		

hours. Fecal coliform counts were estimated using a 3-tube replication of the most-probable-number series (MPN)³⁾. The water samples were diluted with 0.85% NaCl solution, then sprayed into a nutrient agar medium and cultivated for 24 hours at 37°C. The total viable counts (TVC) were then determined by counting the number of colonies formed.

4. Isolation of *V. cholerae* non-O1: The method followed for examining the *V. cholerae* was that of the Japanese society for bacteriology⁴⁾. Immediately after the river water had been sampled, 450 ml was mixed with 50 ml alkaline peptone medium and, after cultivating for six hours, a portion of the supernatant medium was transferred to a non-salt alkaline peptone medium and the remainder streaked onto thiosulphate citrate bile salts agar medium (TCBS, Eiken). More than three colonies were picked up from the TCBS medium. The isolated strains are identified in Table 1.

5. Detection of cholera enterotoxin (CT) from *V. cholerae* non-O1: All strains isolated from the river water were incubated in Syncase medium and the cultivated medium was tested with reversed passive latex hemagglutination (Denka, VET-RPLA kit).

Results

It was expected that, because the environmental conditions of the Sagami R. and the Sakai R. differed, the water quality and composition of organisms would be different. Table 2 shows the range of TVC and coliform and the water quality ranges for each of the locations on the Sagami R. (locations 1—4) and on the Sakai R. (locations 5—9).

At the uppermost location of both rivers, locations 1 and 5, the TVC counts were the lowest. At each of the downstream locations, the TVC counts for the Sagami R. ranged over 10^3 – 10^4 /ml, whereas the Sakai R. recorded a wider range of values with a lower limit of 10^4 /ml and upper limits of 10^5 and 10^6 /ml.

The number of coliform in the Sagami R. showed an uniform increase when going from upstream to downstream and had a range of 10^2 to 10^5 /100 ml. However, for the Sakai R., the number of coliform showed a lower limit of 10^4 /100 ml and an upper limit of 10^7 /100 ml at location 7 but decreased to 10^6 /100 ml at location 8 and 10^5 /100 ml at location 9.

The values and ranges of the water quality parameters differed markedly between the two rivers. That is, on the Sagami R. the concentration of Na⁺ and Cl[−] ions and water hardness increased when going downstream, especially at the lowest location, location 4. However, all locations recorded low values of

Table 2 Range of various environmental parameters at the location of the Sagami River and the Sakai River

Location	Microbiological parameter		Temp. °C	W. temp. °C	pH	Water Quality parameter						
	TVC /ml	Coliform MPN/100ml				DO mg/l	Hardness mg/l	E.C. μ S/cm	Na ⁺ mg/l	Cl ⁻ mg/l	NH ₄ ⁺ mg/l	PO ₄ ³⁻ mg/l
1	4.2×10 ² ~3.9×10 ³ *	9.1×10 ² ~2.1×10 ⁴	13.0 ~19.8	13.4 ~21.8	7.4 ~8.4	5.5 ~7.3	57.2 ~66.4	185.5 ~204.0	2.0 ~10.0	4.8 ~5.6	<0.1 ~0.1	<0.05 ~0.1
2	2.0×10 ³ ~2.0×10 ⁴	9.2×10 ² ~1.1×10 ⁵	19.0 ~30.0	16.7 ~21.2	7.2 ~7.6	6.8 ~7.7	30.8 ~70.3	104.5 ~236.0	0.2 ~20.5	5.2 ~13.5	<0.1 ~2.0	<0.05 ~0.1
3	2.0×10 ³ ~2.5×10 ⁴	2.1×10 ³ ~1.1×10 ⁵	24.0 ~30.5	17.0 ~21.0	7.4 ~7.6	6.4 ~8.1	50.6 ~96.0	118.0 ~249.0	0.6 ~19.0	7.6 ~14.0	<0.1 ~1.0	0.05 ~0.1
4	1.3×10 ³ ~1.1×10 ⁴	9.3×10 ³ ~2.4×10 ⁵	24.0 ~29.5	16.6 ~26.0	6.6 ~7.4	4.4 ~9.4	61.6 ~184.7	137.0 ~419.0	19.0 ~64.0	11.5 ~29.0	<0.1 ~1.0	0.05 ~0.1
5	1.9×10 ⁴ ~2.4×10 ⁵	9.3×10 ⁴ ~2.4×10 ⁵	15.0 ~22.2	13.8 ~18.8	6.4 ~7.4	3.6 ~4.6	87.9 ~91.6	200.5 ~324.5	38.0 ~70.0	13.5 ~19.0	<0.1 ~0.5	0.05 ~0.3
6	5.8×10 ⁴ ~2.2×10 ⁵	4.4×10 ⁴ ~2.4×10 ⁶	13.8 ~24.5	14.0 ~20.4	6.0 ~7.4	3.2 ~5.2	109.9 ~149.8	413.5 ~1065	180 ~970	39.0 ~154	3.0 ~>5.0	0.5 ~2.0
7	6.7×10 ⁴ ~1.8×10 ⁶	2.9×10 ⁴ ~1.1×10 ⁷	8.0 ~30.0	10.9 ~26.2	6.2 ~7.4	2.6 ~5.0	105.5 ~127.5	359.5 ~757.5	180 ~420	41.0 ~71.0	<0.1 ~>5.0	0.7 ~3.0
8	6.4×10 ⁴ ~1.4×10 ⁶	2.1×10 ⁴ ~2.4×10 ⁶	11.0 ~31.0	12.0 ~25.8	6.0 ~7.4	4.1 ~6.6	96.7 ~116.5	355.0 ~605.0	180 ~305	47.0 ~63.0	<0.1 ~>5.0	0.7 ~5.0
9	4.2×10 ⁴ ~2.4×10 ⁵	2.0×10 ⁴ ~4.3×10 ⁵	9.0 ~29.0	10.6 ~25.4	6.8 ~7.4	3.1 ~6.2	96.7 ~145.1	372.0 ~568.0	170 ~280	44.5 ~58.0	<0.1 ~>5.0	0.3 ~2.0

Abbreviation ; TVC : total viable counts, Coliform : fecal coliform (most probable number), Temp. : air temperature, W. temp. : water temperature, DO : dissolved oxygen, E.C. : electric conductivity, Na⁺ : sodium ion, Cl⁻ : chloride ion, NH₄⁺ : ammonia nitrogen, PO₄³⁻ : phosphate ion.

*minimam~maximam range.

Table 3 Seasonal variation in *Vibrio cholerae* non-O1 was isolated at location 9

Date ¹⁾	Point of river	Temp. °C	W. temp. °C	TVC /ml	Cl ⁻ mg/l
7/09	C ²⁾	29.0	25.2	1.8×10 ⁵	50.5
9/17	B	25.0	22.7	1.6×10 ⁵	55.0
10/03	C	26.0	21.8	5.2×10 ⁴	55.5
11/01	B	19.0	17.3	1.9×10 ⁵	45.5
12/03	N	9.0	10.6	4.5×10 ⁴	55.5

Abbreviation ; 1) Date : sampling date, Point of river : *Vibrio cholerae* non-O1 was isolated from the point of river, Temp. : air temperature, W. temp. : water temperature, TVC : total viable count, Cl⁻ : concentration of chloride ion, 2) C : center of river, B : both of center and edge of river, N : not isolated

NH₄⁺ and PO₄³⁻. For the Sakai R., the lowerbounds of ion solute concentration recorded for each of the locations were almost constant, excluding the uppermost location 5, but the upper bounds recorded were larger depending on the location. When comparing the ion solute concentrations for both rivers, the Sakai R. showed a larger range than that shown for the Sagami R.. Although it is not shown in the table, the salinity concentrations at all locations had the low of value of 0~2‰, and the degree of transparency measured at locations 7, 8, 9 on the Sakai R. was approximately 20~30 cm but at all other locations was greater than 30 cm.

There was no difference in water temperature between the two rivers. In July the temperature was approximately 25°C and in winter, in December, it decreased to approximately 11°C. This trend, typified by location 9, is shown in table 3 with the values being calculated from the mean for each sample. As stated above, the range of values found for the water quality experiments and TVC and coliform tests was small, and the level of pollution increased when going from upstream to downstream. However, at each of the locations on the Sakai R. the degree of pollution differed and, compared with the Sagami R., the number of

Table 4 *Vibrio cholerae* non-O1 was isolated from the point of river site and ratio of isoation

River	Location	Ratio of isolation		
		The point of river site		Location total
		Edge	Center	
Sagami R.	1	0/ 3 ¹⁾	0/ 3	0/ 6
	2	1/ 4	1/ 4	2/ 8
	3	2/ 4	4/ 4	6/ 8
	4	3/ 4	1/ 4	4/ 8
	total	6/15	6/15	12/30
Sakai R.	5	ND ²⁾	0/ 3	0/ 3
	6	2/ 3	1/ 3	3/ 6
	7	2/ 5	2/ 5	4/10
	8	1/ 5	4/ 5	5/10
	9	2/ 5	4/ 5	6/10
	total	7/18	11/21	18/39

1) number of isolated *Vibrio cholerae* non-O1/number of samples

2) ND: not done

Table 5 Environmental microbiology and water quality parameter with or without isolation of *Vibrio cholerae* non-O1 in the Sagami River and the Sakai River

River	isolation	Microbiological Parameter ¹⁾		Water Quality Parameter ²⁾						
		TVC /ml	Coliform MPN/100ml	DO mg/l	hardness mg/l	E.C. μS/cm	Na ⁺ mg/l	Cl ⁻ mg/l	NH ₄ ⁺ mg/l	PO ₄ ³⁻ mg/l
Sagami	with	(1.1±0.7)×10 ⁴ *	(4.0± 6.7)×10 ⁴	6.7 ±1.1	83.5 ±33.7	220.5 ± 81.3	21.1 ± 15.9	14.0* ± 5.6	0.2 ±0.3	0.08* ±0.03
	without	(5.4±5.4)×10 ³	(1.9± 2.6)×10 ⁴	7.1 ±0.8	66.9 ±20.7	195.8 ± 73.9	12.1 ± 14.0	8.5 ± 4.0	0.2 ±0.5	0.05 ±0.03
Sakai	with	(3.0±3.2)×10 ⁵	(1.0± 2.5)×10 ⁶	4.7 ±1.0	116.8 ±13.9	479.5 ±116.6	260.8 ± 63.4	53.6 ± 7.3	4.0 ±2.2	1.24 ±0.65
	without	(3.4±5.1)×10 ⁵	(6.7±11.1)×10 ⁵	4.2 ±1.0	119.3 ±17.0	505.7 ±212.2	274.4 ±185.9	52.6 ±27.1	3.0 ±2.4	1.35 ±1.11

1) (mean±S.D.), 2) mean±S.D.,

Abbreviation ; TVC: total viable counts, Coliform: fecal coliform (most probable number)., DO: dissolved oxygen., E.C.: electric conductivity., Na⁺: sodium ion., Cl⁻: chloride ion., NH₄⁺: ammonia nitrogen., PO₄³⁻: phosphates ion.

*statistic significance (p<0.05)

TVC and coliform were greater, as was the ion solute, indicating that the river was very polluted.

As shown in table 4, of the 69 water samples collected from both rivers during this survey, 30 (43.5%) were isolated to contain *V. cholerae* non-O1. The percentage isolated for the Sagami R. was 40.0% (12/30) and that for the Sakai R. was 46.2% (18/39). *V. cholerae* non-O1 was not isolated at the uppermost locations of either rivers (locations 1 and 5), but for both rivers, the percentage isolated increased when going downstream. At location 7, 8, 9 on the Sakai R., when the water temperature was below 11°C *V. cholerae* non-O1 was not isolated at all but when the water temperature increased to above 11°C it was always isolated at locations 8 and 9.

At each location (excluding the location 5) on the river the percentage isolated in running water at the center of river and in standing water at the river's edge was 51.5% (17/33) and 39.4% (13/33) respectively. There was no significant difference shown between the isolation of this organism in running water at the center of the river and isolation in standing water at edge of the river for either of the rivers.

The samples of river water which were detected to contain *V. cholerae* non-O1 (from locations 6, 7, 8 of the Sakai R.) produced a CT strain with a ratio of 4/30 (13.3%).

The microbiological and water quality parameters recorded from the water samples with and without isolation of *V. cholerae* non-O1 are shown in table 5. For the Sagami R. it was noted that TVC and the ion Cl^- and PO_4^{3-} were greater when *V. cholerae* non-O1 was detected. However, for the Sakai R., no difference was observed in the water quality parameters with or without isolation of *V. cholerae* non-O1. Although all the water quality parameters have higher values for the Sakai R. than those for the Sagami R. it does not necessarily follow that *V. cholerae* non-O1.

Discussion

The occurrence of *V. cholerae* O1 in rivers is sometimes reported but it is usually short-lived. However, *V. cholerae* non-O1, which is of the same group as O1, can be isolated at the same time that O1 is prevalent and also after such outbreaks have occurred¹⁵⁾. Furthermore, Colwell R.R. et al. interpreted that there was a possibility that *Vibrio* spp. can exist as bacteria in rivers and other aquatic environments⁶⁾. That is, when *V. cholerae* non-O1 occurs, growth is possible, given the right environmental conditions, and it is possible that, through this polluted water and aquatic foods, people may become infected⁷⁾. Reports on infection from aquatic foods and reports on isolation in environmental survey have focused on salinity concentrations⁸⁾⁹⁾. In the survey conducted at Chesapeake Bay, it was found that *V. cholerae* non-O1 can exist in concentrations of 4~17‰ salinity²⁾ conversely, existence is also reported for clear rivers such as the Western Colorado R. which has an Na^+ concentration of less than 5 mM¹⁰⁾.

In our results for the Sagami R., the Na^+ concentration was about 20 mg/l (Na^+ concentration approx. 1 mM) when *V. cholerae* non-O1 was isolated but there was no significant difference shown with Na^+ concentration when not isolated although the results were slightly higher. At each location the salinity concentration was 0~2‰ (salinity of concentration 0.04~0.3‰ when converted to Cl^- concentration) therefore we do not consider salinity concentrations to be a suitable indicator for the existence of *V. cholerae* non-O1.

In this survey the rivers were, for comparison purposes, two of differing environments with many natural features, such as the characteristic water quality and composition of aquatic microbial flora, and we investigated the effect these had on the existence of *V. cholerae* non-O1 and from these results we considered that it may be possible for the water quality characteristics to be used for an indicator for showing existence.

It is usual for water samples from many different locations to be mixed together and examined, but in our investigation we individually examined the water samples taken from running water at the center of the river and from standing water at the edge of river for each of the locations. Our results showed a high frequency of isolation at the center of running water at several locations but there was no significant difference shown in the frequency of isolation between the center and edges. These results suggest that, not only does *V. cholerae* non-O1 flow in from polluted areas and float down fast flowing water, but also exists widely at each location.

It is reported that the isolation of *V. cholerae* non-O1 changes seasonally and, in particular, is influenced by low temperatures¹¹⁾. At location 7, 8 and 9, of the five times surveyed, excluding the time in December when the water temperature was below 11°C, isolation occurred at either the center or the edge of the river at each of these locations (in July no isolation at location 7). However, when the water temperature dropped to 11°C *V. cholerae* non-O1 was not isolated from any of the water samples. As it can be seen from the results for location 9 (Table 3) a decrease was shown for TVC but there was no observed change in the water quality, so we believe the reason why isolation did not occur below 11°C was because of the effect of

the drop in temperature.

For the Sakai R., excluding the samples that were influenced by low water temperatures (under 11°C) during the winter season, the isolation rate was 50% (4/8) at location 7, 62.5% (5/8) at location 8, 75% (6/8) at location 9 and the isolation rate for all the samples taken from the Sakai R. was 54.5%. When compared with the Sagami R. the isolation rate was higher, even when disregarding the effect of fall in temperature. Furthermore, as the level of pollution increased when going downstream, the rate of isolation of this organism also increased for both rivers. This indicates that higher pollution levels make existence of *V. cholerae* non-O1 possible.

V. cholerae non-O1 was not detected at all throughout the period of the survey at locations 1 and 5. Both these locations were situated at the uppermost part of the rivers where the pollution levels for both rivers were the lowest. Even at location 5 on the Sakai R. where the width was less than 1 meter, the water volume extremely small and the level of pollution higher than that for the Sagami R. *V. cholerae* non-O1 was not isolated. It is assumed that this was because at this uppermost location there was no scattering of this organism caused by the tide flowing upstream and the inflow of polluted water was also small, but it may be assumed that, because of the level of pollution.

V. cholerae non-O1 may exist in the future. However at present, even if this organism were to occur, it is very unlikely to exist because of some other primary factor. That is, different microbial flora in differing water qualities at each location and in each river interact with *V. cholerae* non-O1¹²⁾ and thus it is considered that this has an effect on the existence of this organism.

For the Sagami R. isolation of *V. cholerae* non-O1 was significant when the TVC counts were high and when the concentrations of Cl^- and PO_4^{3-} were high. Correlation was shown between Cl^- and Na^+ , and Cl^- and EC, but the values for Na^+ and EC were spread over a wide range and a statistical difference, like that found for Cl^- was not shown. This suggests that Cl^- is a better indicator for the isolation of *V. cholerae* non-O1.

According to Hood M.A. et al. the isolation of *V. cholerae* non-O1 is not necessarily correlated to coliform¹³⁾, which is used as an indicator in aquatic environments contaminated with human and animal feces, so it cannot be used as an indicator for the isolation of *V. cholerae* non-O1 and thus this organism does not derive from human and animal feces. In our survey we also found the relation between coliform and *V. cholerae* non-O1 to be unclear so this suggested that *V. cholerae* non-O1 did not derive from human and animal feces.

In this survey the sampled river water was transported at room temperature (about 20°C). Within 4 hours of sampling, the water was examined and thus the mutual effects on the bacteria in the water were considered to be small but in the future the effects of other organisms and solutes should be considered.

In the past *V. cholerae* O1 has occurred in the Sagami R.¹⁴⁾. Therefore it is quite logical to think that it is also possible for *V. cholerae* non-O1, of the same group, to exist. If the degree of pollution for the Sagami R., as measured by the indicators TVC, Cl^- and PO_4^{3-} , increases to above the range then, we believe that it will be possible for *V. cholerae* non-O1 to exist and increase and it will always be detectable.

Williams L.A. et al. reported that when the quantity and frequency of *V. cholerae* non-O1 increased on the coast, reported cases of infection also increased¹⁵⁾. This implies that if the pollution of the Sagami R. is kept below the regular range then it will be impossible for *V. cholerae* non-O1 to exist or increase and cases of human infection will become fewer.

For the Sakai R., the relationship between the isolation of *V. cholerae* non-O1, water quality and the number of bacteria was not clearly defined. But, no matter what the conditions, the rate of isolation was high at all of the locations, so we believe that *V. cholerae* non-O1 is capable of existing like microbial flora.

It is reported that enterotoxins are produced from strains of *V. cholerae* non-O1 isolated from the

environment¹⁶⁾ and, in our survey, we also isolated the CT strain. The percentage isolated was 5.8% (4 of the 69 samples) and 13.3% from the samples with *V. cholerae* non-O1 isolated, which is very low when compared with the rate of isolation of this toxin from Chesapeake Bay²⁾ or from the Colorado R.¹⁰⁾. Moreover, it is considered that there is little chance of infection from the toxin-producing strain as aquatic foods such as fish and shellfish are not taken from the Sakai R.. On the other hand, for the Sagami R., the possibility of its existence cannot be ignored even though strains exhibiting CT were not detected. Thus if the pollution level increases or, by some change in the environment, the strain exhibiting CT grows, then we must pay special attention to this as the risk of infection through *V. cholerae* non-O1 will also increase.

From the above, we believe that it is desirable to keep the level of pollution below the regular level as much as possible and to use the indicators TVC, Cl^- , and PO_4^{3-} , in order to detect and prevent the growth of *V. cholerae* non-O1.

Acknowledgements

The authors are greatly indebted to Mr. Satoru Takaoka, Mr. Toshihiro Ishikawa and Mr. Atsushi Hagiwara (trainees of the Department of Public Health, School of Hygienic Sciences, Kitasato University) for the investigation of the rivers.

The substance of this paper was reported at the conference of the 47th Japanese Society of Public Health (Sapporo).

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河川水質と *Vibrio cholerae* non-O1の検出について

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(平成1年3月1日受付)

(平成1年5月2日受理)

自然環境から *Vibrio cholerae* non-O1が検出されたという従来の報告によれば、検出が海水か河川水などかによって、検出時の環境の個々の条件が異なっている。

そこで、2つの河川で河川水質の差異、および指標細菌の動向を中心に *V. cholerae* non-O1の生存条件の検討を試みた。

1) *V. cholerae* non-O1は陸上や他の汚染地点からの流入ではなく、河川のその検出地点での生存が考えられた。

2) 水質汚染度の高い河川、あるいは同一河川で

も水質汚染度の高い地点ほど *V. cholerae* non-O1の検出率が高かった。

3) 水質汚染度の低い河川あるいは水質汚染度の変動の少ない河川でC1⁻イオン、リン酸イオンの増加、または普通寒天培地上37℃24時間で集落を形成する細菌菌数の増加は *V. cholerae* non-O1の検出度を高めるようである。

4) *V. cholerae* non-O1の検出させた試料のうちコレラエンテロトキシン産生菌を含む試料は約13%であった。