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Biological Properties of *Vibrio cholerae* Isolated from the Philippines in 1985

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Abstract: Sixty strains of *Vibrio cholerae* isolated from the Philippines in 1985 were examined for their biological properties and sensitivity to eight kinds of antimicrobial agent. By the serotyping, 58 strains (96.6%) and one strain (1.6%) were Ogawa and Inaba-type respectivery, but one strain (1.6%) was not agglutinated by diagnostic antiserum. This strain was identified as non Ol (NAG) vibrio supported by necessary biochemical characters. For the biotyping, 59 strains of *V. cholerae* Ol were resistant to phage IV and polymixin B, and positive for chicken red blood cell agglutination and VP reaction. And all strains showed hemolytic activity to the sheep red blood cell using Zinnaka's modified method. Therefore, these strains belong to biovar *eltor*. In prophage typing, 53 strains (89.8%) were Celebes-original type and remaining six were Celebes-cured type. Regarding the sensitivity test to drugs, chloramphenicol, tetracycline and nalidixic acid were the most effective drugs and the secondary effective drugs were ampicillin and erythromycin. MICs of all strains were below 12.5 mcg/ml for kanamycin and 25 mcg/ml with cephaloridine, but MICs for streptomycin were rather high. Neither tetracycline-resistant *V. cholerae*, such as Tanzania, Bangladesh and Kenya nor multiple drug resistant *V. cholerae* was found. In addition to those above, O/129 resistant *V. cholerae* reported by some researchers was not detected in this investigation.

Key words: *Vibrio cholerae*, Biological property, Epidemiology, Drug sensitivity

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INTRODUCTION

The seventh cholera pandemy by *eltor* type of *Vibrio cholerae* originated from Celebes Island, Indonesia in 1961 were still spreading around the tropics, such as Southeast Asia, Indian subcontinent and African countries. Studies on the geographical and chronological differences in properties of enteropathogens, especially biological properties of *V. cholerae* including serotype, prophage type, phage type of *eltor* vibrio and drug sensitivity, are continuing in our laboratory, comparing the isolates from the Philippines as Southeast Asia and Kenya as East Africa (Naito *et al.*, 1977; Iwanaga *et al.*, 1979; Iwanaga and Mori, 1981; Iwanaga *et al.*, 1982; Mori, 1983; Ichinose, 1985). The present report describes the biological properties and drug sensitivity of *V. cholerae* biovar *eltor* isolated from inpatients in the Philippines in 1985.

MATERIALS AND METHODS

Strains: As shown in Table 1, 60 strains were used for the present study. Out of 60 strains, 42 were isolated from inpatients at Research Institute for Tropical Medicine, Manila, and kindly provided by Dr. Yoshinori Kaneko, Director of the Institute. Remaining 18 strains were isolated from inpatients at San Lazaro Hospital, Mania, and provided by Dra. Santiago in the Hospital. All strains were inoculated in the butt of semi-solid nutrient agar (Eiken) and brought back to Japan by Y. Sakata and her colleagues of medical student.

As a sensitive indicator for κ phage, strain H218 stocked in our laboratory was employed. As control strains for biotyping, classical 82Bg15 isolated in Bangladesh in 1982 and supplied by Dr. Yoshifumi Takeda, Research Institute for Microbial Diseases, Osaka University (present adress: Institute of Medical Science, The University of Tokyo), and *eltor* 82P6, isolated at San Lazaro Hospital, Manila in 1982 were used. These three strains were maintained in butt of semi-solid nutrient agar in our laboratory.

Tests for biochemical properties: At the first step, all strains were inoculated to TCBS agar (Eiken) and confirmed the fermentation of sucrose. Gram-staining, oxidase test

Table 1. *Vibrio cholerae* strains tested

Strain No.	No. of strains	Place isolated	Period isolated
85P1–85P42	42	RITM*	Feb.–Aug., 1985
85P43–85P60	18	SLH**	Aug., 1985

*: Research Institute for Tropical Medicine, Manila, the Philippines.
**: San Lazaro Hospital, Manila, the Philippines.

(Nissui paper strip) and serotyping by *V. cholerae* antiserum (Denka Seiken) were performed from overnight growth on nutrient agar. Using Kligler's iron agar, SIM agar, VP semi-solid agar (Sasagawa-Ikemura's VP agar), lysine decarboxylase broth (Falkow's formula) and Simmons citrate agar (all media were Eiken's products), all available properties were recorded. Kovac's reagent and Barritt's reagent were used for indol production in SIM culture and acetoin production from dextrose (VP reaction) respectively.

In the cases of lysine decarboxylase broth, sterilized liquid paraffin was immediately overlaid soon after inoculation to protect the natural alkalization of media.

Adding to above, ornithine decarboxylation, arginine dehydrolation, fermentation of inositol and mannitol, and growth in 0% sodium chloride pepton water were tested if test organisms did not agglutinate with antiserum.

Detection of biotype: Sensitivities to phage IV for classical *V. cholerae* and polymixin B, agglutination of chicken red blood cell (CRBC), hemolysis of sheep red blood cell (SRBC) and Voges-Proskauer (VP) reaction were used for detection of biotype. In general, the original methods for each test with slight modification were applied.

For phage IV (Mukerjee, 1963) and polymixin B (Gangarosa *et al.*, 1967) tests, strains inoculated in 3 ml of pepton water (1% polypepton, 0.3% yeast extract, 0.5% NaCl, pH 7.2) were incubated at 37°C for 18 hours. Then, the cultures were diluted to 10 fold by physiological saline (PS), and 0.1 ml of diluted cultures were mixed with 3 ml of semi-solid agar (pepton water plus 0.8% agar), and overlaid on 15 ml of pepton agar (pepton water plus 1.6% agar) plates. Twenty microliter of 1 RTD (10^5 PFU/ml) phage IV solution was spotted by pipett tips using Pipetman (Gilson), and 50 u polymixin B discs (Eiken) were also placed on the same overlaid plate, and incubated at 37°C for 18 hours.

For CRBC agglutination test (Finkelstein and Mukerjee, 1963), well washed fresh CRBC was suspended in PS by the concentration of 2.5 per cent. Slideglass agglutination tests were carried out using 18 hr growth on nutrient agar.

For SRBC hemolysis test, Zinnaka's modified method (1981) originated from Feeley and Pittman's method (1963) was used. For the comparative study, strains were incubated in 2 ml of heart infusion broth (Eiken) at 37°C for 24 hr (Zinnaka, 1981) and 18 hr (Ichinose, 1985) without shaking. Two-tenth milliliter of 5% SRBC-PS suspension was added to the culture, then incubated at 37°C for two hours. After that the mixtures were kept in refrigerator overnight, then the degree of hemolysis was recorded. The degree of hemolysis (Ichinose, 1985) were as follows; ++: complete hemolysis, +: hemolysis with a small quantity of SRBC sediment at the bottom of test tube, ±: weak hemolysis with a large quantity of SRBC sediment, and −: completely no hemolysis.

Test for prophage types: The lysogenicity test (Takeya and Shimodori, 1963; Zinnaka *et al.*, 1966) for κ phage were applied. For the test, organisms were incubated in 3 ml of

pepton water at 37°C for 18 hr without shaking. Then, cultures were vigoroughly shaken with two drops of chloroform and centrifuged 3,500 rpm for 40 minutes. On the other hand, an indicator strain H218 was cultured in pepton water at 37°C, 18 hours. One-tenth milliliter of H218 culture which was diluted to 10 fold with PS, and 3 ml of semi-solid pepton agar (without yeast extract) were mixed thoroughly, and overlaid onto 15 ml of previously poured pepton agar (without yeast extract). One drop (20 μl) of supernatant from each chloroform-treated culture was spotted on plates mentioned above. The presence of κ phage was confirmed by lysis of indicator after incubation at 37°C overnight. When κ phage was not detected, sensitivity to κ phage test (Takeya *et al.*, 1965) was carried out. Eighteen hour cultures at 37°C of strains to be tested were diluted to 10 fold by PS, and 0.1 ml of them and 3 ml of semi-solid agar were mixed, and overlaid to 15 ml of previously poured pepton agar. One drop (20 μl) of κ phage solution, supernatant of 85P49 (selected by preliminary test) culture, was spotted on the overlaid plate, and incubated at 37°C overnight. The lysis of test strains indicated the sensitivity to κ phage.

O/129 sensitivity test: The vibriostatic agent, 2,4-diamino-6,7-diisopropyl-pteridine (O/129) sensitivity test was performed by the method of Shewan *et al.* (1954). One hundred milligram of O/129 phosphate (Sigma Co.) was dissolved in 100 ml of distilled water, and absorbed to Whatman AA discs (6 mm diamater), then discs were dried at 37°C. One disc absorbes about 20 μl of solution, so each disc contains 20 mcg of O/129.

Drug sensitivity test: Sensitivity to antimicrobial drugs was tested by determining minimal inhibitory concentrations (MICs) according to the standard agar dilution method recommended by the Japan Society of Chemotherapy (1981). Strains were cultured in heart infusion broth at 37°C overnight, and their 100 fold dilutions (10^6 cells/ml) were inoculated on heart infusion agar (Eiken), containing 2 fold serial dilutions of the drugs, starting from 100 mcg/ml to 0.2 mcg/ml. Eight kinds of antimicrobial drugs (Sigma Co.) tested were tetracycline (TC), chloramphenicol (CP), nalidixic acid (NA), ampicillin (AB-PC), kanamycin (KM), erythromycin (EM) and cephalorydine (CER). After incubation at 37°C for 18 hr, the growth of strains to be tested were observed. The MICs were defined as the lowest concentration of the drugs at which showed the complete inhibition of the growth.

RESULTS

Biochemical properties: The results of biochemical tests, including oxidase, O/129 sensitivity and Gram's staining of 60 strains are shown in Table 2. As a result, all strains showed completely the same character, including sensitivity to vibriostatic agent O/129.

Serotypes: Serotypes detected are shown in Table 3. Out of 42 strains isolated from RITM, 41 strains were Ogawa-type and remaining one was Inaba-type. On the other

hand, out of 18 strains isolated from SLH, 17 strains were Ogawa-type, but a remaining strain 85P59 did not agglutinate with any anti-cholera serum applied. Also this strain was negative for agglutination using cells treated with 121°C for 30 minutes. The results of additional biochemical properties for this strain are as follows: ornithine decarboxylation (+), arginine dehydrolation (−), fermentation of mannitol (+), fermentation of inositol (−), growth in 0% sodium chloride pepton water (+). Consequently, this strain was identified as non O1 *V. cholerae* (so-called NAG vibrio).

Biotypes: Results of the tests for deciding biotypes, classical or *eltor*, were summarized in Table 4. All *V. cholerae* O1 strains and one strain of NAG vibrio showed resistance to

Table 2. Biochemical properties of strains tested

Properties	Results	Frequency
Sucrose on TCBS agar	Positive	60/60
Kligler's iron agar		
Lactose	Negative (1 day)	60/60
Dextrose	Positive	60/60
Gas from dextrose	Negative	60/60
Hydrogen sulfide	Negative	60/60
SIM agar		
Indol reaction	Positive	60/60
Motility	Positive	60/60
IPA reaction	Negative	60/60
Hydrogen sulfide	Negative	60/60
VP reaction	Positive	60/60
Lysine decarboxylase	Positive	60/60
Simmons citrate	Positive	60/60
Oxidase test	Positive	60/60
O/129 (20 mcg/disc) test	Sensitive	60/60
Gram's staining	Gram negative rods	60/60

Table 3. Serovars observed in *V. cholerae* tested

Serovar	Place isolated		Total
	RITM	SLH	
Ogawa	41	17	58 (96.6%)
Inaba	1	0	1 (1.6%)
non O1 (NAG)	0	1	1 (1.6%)
Total	42	18	60

phage IV and polymixin B, and positive reaction for CRBC agglutination and VP tests. All were typical characters of biovar *eltor*.

Table 4. Results of the tests related to biotyping of *V. cholerae* Ol

Tests	Number of the strains in	
	RITM	SLH
Phage IV sensitivity		
resistant	42	17
sensitive	0	0
Polymixin B sensitivity		
resistant	42	17
sensitive	0	0
CRBC agglutination		
positive	42	17
negative	0	0
VP reaction		
positive	42	17
negative	0	0

One strain of NAG vibrio isolated from SLH showed the same results.

Table 5. Number of strains showing each degree of hemolysis

Degree of hemolysis		Experiment 1 18 hr culture	Experiment 2 24 hr culture	Experiment 3 24 hr culture
Marked hemolysis	(++)	1	0	0
Hemolysis with sediment	(+)	4	58	59
Weak hemolysis	(±)	52	1	0
No hemolysis	(−)	2	0	0
Incidence of hemolytic strains (++ and +)		5/59 8.4%	58/59 98.3%	59/59 100%

Tested by Zinnaka's modified method (1981), originated from Feeley and Pittman (1963). NAG vibrio showed: (+) in Exp. 1, (±) in Exp. 2, (+) in Exp. 3. Control strain 82Bg15 (classical), and HIB-not inoculated showed no hemolysis in every time.

Table 6. Results of prophage typing of *V. cholerae* Ol biovar *eltor*

Type	Place isolated		Total
	RITM	SLH	
Celebes-			
original	40	13	53 (89.8%)
cured	2	4	6 (10.1%)
Total	42	17	59

Hemolysis to sheep red blood cell: The first experiment of hemolysis was performed using the cultures at 37°C for 18 hours. Results obtained were as follows; −: 2/59 (3.3%), ±: 52/59 (88.1%), +: 4/59 (6.7%), ++: 1/59 (1.6%), and a NAG vibrio showed (+). One week later, incubation period was extended to 24 hr and hemolysis was tested. Results were; ±: 1/59 (1.6%), +: 58/59 (98.3%). Another one week later, the third experiment was carried out by using 24 hr culture again, all strains showed +. One strain of NAG vibrio showed ± in the second experiment and + in the third. In the cases of control strain 82Bg15 (classical) and HIB not inoculated showed no hemolysis every time, and control 82P6 (*eltor*) showed ± in the first experiment, and + in other two experiments (Table 5).

Prophage types: Fifty-nine strains of biovar *eltor* were tested for prophage types (Table 6). Among them, 40 strains from RITM and 13 strains from SLH were found to liberate the κ phage, so that these strains belong to Celebes-original type. Remaining two strains from RITM and four from SLH were not lysogenic for κ phage and sensitive to κ phage. Then, they were dicided to Celebes-cured type. For reference, one NAG vibrio did not liberate κ phage and insensitive to κ phage.

Drug sensitivity: Table 7 indicates the number of *V. cholerae* Ol isolates showing each MIC for antimicrobial drugs. The commonest MICs were 0.78 mcg/ml (33 strains, 55.9 %) and 1.56 mcg/ml (21, 35.5%) to CP, 50 mcg/ml (47, 79.6%) to SM, 0.78 mcg/ml (24, 40.6%) and 0.39 mcg/ml (21, 35.5%) to TC, 0.2 mcg/ml or less (58, 98.3%) to NA, 1.56 mcg/ml (51, 86.4%) to AB-PC, 6.25 mcg/ml (41, 69.4%) and 12.5 mcg/ml (18, 30.5%) to KM, 1.56 mcg/ml (35, 59.3%) and 3.13 mcg/ml (24, 40.6%) to EM and 25 mcg/ml (59, 100%) to CER.

Table 7. Distribution of MICs for 59 strains of *Vibrio cholerae* Ol

MIC (mcg/ml)	CP	SM	TC	NA	AB-PC	KM	EM	CER
less than or 0.2	2		5	58				
0.39	3	1	21	1				
0.78	33		24		5			
1.56	21		8		51		35	
3.13		1	1		3		24	
6.25						41		
12.5		5				18		
25		4						59
50		47						
100		1						
more than 100								

MICs of one strain of NAG vibrio were as follows:
CP≦0.2, SM 12.5, TC≦0.2, NA≦0.2 AB-PC 0.78, KM 3.13, EM 1.56 and CER 12.5 mcg/ml.

DISCUSSION

Regarding biochemical properties and O/129 sensitivity in Table 2, all strains showed the same biological character of *V. cholerae*. These results satisfied the minimal criteria to identity *V. cholerae* defined by Ministry of Health and Welfare, Japan (1980). Shewan *et al.* (1954) reported the usefulness of O/129 sensitivity test for differentiation between *V. cholerae* and related bacteria, especially Genus *Aeromonas*. Recently, Sundaram and Murthy (1983) and Matsushita *et al.* (1984) reported the O/129 resistant *V. cholerae*. The latters suggested that O/129 resistance of *V. cholerae* closely associated with trimethoprim-resistance in the same plasmid encorded. Also Ichinose (1985) reported resistant strains isolated from Kenya during 1983 to 1984 and from the Philippines in 1982 and 1984, having the resistant rates of 252/288 in Kenya and 1/29 in the Philippines. He warned the invalidity of O/129 sensitivity test for differentiation of *Vibrio* species from other gram-negative bacilli any more. Nevertheless isolates in this investigation were completely sensitive to O/129.

Concerning the serotypes, the predominant serovar was Ogawa (58/60, 96.6%), and Inaba type was only one (1.6%). Another strain did not agglutinate to polyvarent anti-cholera serum, even after heat treatment. Additional biochemical properties for this strain coincide the character of *V. cholerae*, so that this one must be so-called NAG vibrio (Ministry of Health and Welfare, 1980).

In the Philippines, the predominant serovar was Ogawa in 1964 like 5,962/5,995 and 601/606 from two different laboratory (Gomez *et al.*, 1967), and in 1982 and 1984 showing 28/29 (Ichinose, 1985). According to the report of Kenyan strains, the predominant serovar was Inaba in 1974/75 (Naito *et al.*, 1977), but it schifted to Ogawa-type (Iwanaga and Mori, 1981; Iwanaga *et al.*, 1982; Mori, 1983; Ichinose, 1985).

To differentiate classical and *eltor* biovars in *V. cholerae* Ol, it has been supported usefulness of classical cholera phage IV and polymixin B sensitivity test, CRBC agglutination test, VP reaction and SRBC hemolysis test. Former two tests are satisfactory methods for differentiation of biotypes, although others were showing varied results discribed by many researchers. For example, Zinnaka (1981) pointed out the existence of classical strains stocked for long period showing positive reaction in CRBC agglutination test, classical strains having weak positivity in VP reaction and non hemolytic *eltor* strain especially isolates after 1963. Even in polymixin B sensitivity test, an atypical result, a sensitive *eltor* strain, was reported by Ichinose (1985). However, all strains tested in this study showed same results except SRBC hemolysis test (Table 4).

About SRBC hemolysis test, fundamental study by Feeley and Pittman (1963) demonstrated that hemolysin producted by *eltor* vibrios disappered more quickly. And hemolysin is probably destroyed by certain other products of the vibrios, particularly when there is an alkaline schift in media, especially in traditional method (by Greig, 1914, using alkaline broth culture in 37°C for 3 days). They also indicated that the optimum

pH of the media for hemolysin production was 7.1 to 7.4. To prevent the alkaline schift resulting in the growth of vibrio in the media, Barua and Mukherjee (1964) added one per cent of glycerol to heart infusion broth (HIBG) and brain heart infusion broth, expecting that glycerol possibly act as a stabilizer of pH. Barua and Gomez (1967) compared three kinds of culture from strains in the Philippines during 1961 to 1965. The percentage of strains showing hemolytic activity decreased year by year in all media. They recommended the HIBG, but negative strains for hemolysis remained in 1964 (15% in 893 strains) and in 1965 (17% in 611) comparing with 100% hemolysis in 1961, 1962 and 1963. Sakazaki *et al.* (1971) confirmed the reproducible result using the HIBG, and also satisfactory result using an aerobic plate of brain heart-thioglycollate-cystine agar. However, Zinnaka (1981) criticized HIBG method, because hemolysin was detected even in classical-type vibrios.

Since the African continent was invaded by *eltor* cholera pandemy in early 1970, the most of strains did not show hemolytic activity (Barua and Burrows, 1974). Iwanaga *et al.* (1982) found the recovery of hemolytic activity in 7 out of 64 strains (11%) showing marked hemolysis in 1975, and in 210 out of 272 strains (77%) isolated during 1980 to 1981, and Mori (1983) reported marked hemolysis in 324 out of 434 strains (74.7%) isolated during 1980 to 1982 in Kenya. Ichinose (1985) described that 75.5% in West Kenya and 77.1% in Central Kenya isolated in 1983 and 78.3% in West Kenya in 1984 showed hemolytic activity in heart infusion broth (HIB). On the other hand, in the Philippines, positive rates of hemolytic in Feeley and Pittman's method and HIB were 96% in 1961, 96% in 1962, 28% in 1963, 1.7% in 1964 and 1.1% in 1965 (Barua and Gomez, 1967). Moreover, Ichinose (1985) reported the positive rates for isolates from the Philippines, using HIB and Zinnaka's modified method by 18 hr culture, 5% in 20 strains in 1982, 0% in nine strains in 1984. In this investigation using Zinnaka's modified method, the incidence of hemolytic strains (++ and +) were 8.4% by 18 hr culture, but positive rates were increased to 98.3% (58/59) and 100% (59/59) in duplicated tests using 24 hr culture. This result indicated that there is a complete recovery of the hemolytic activity in the Philippines in 1985.

With regard to prophage types, Iwanaga *et al.* (1981) suspected the routes of cholera spreading in 1980 and 1981 using serotype and prophage types of *V. cholerae* isolated in Kenya. Mori (1983) reported that the predominant prophage type was Celebes-original (369/434, 85.0%) followed by 14.5% (63/434) of Celebes-cured type and classic-Ubol type was only 0.5% (2/434) during 1980 to 1982 in Kenya. Ichinose (1985) described that the most of strains isolated during 1983 to 1984 in Kenya were Celebes-original (337/338, 99.7%) and only one strain was Celebes-cured. Also his comparative study indicated that prophage types of the strains isolated from the Philippines were Celebes-original (17/20, 85%) and classic-Ubol (3/20, 15%) in 1982, and Celebes-original (3/9, 33.3%), Celebes-cured (6/9, 66.6%) in 1984. According to the report of Ohashi *et al.* (1981), prevalence of Celebes type strains in Southeast Asian countries were very

low, especially in Malaysia and Thailand during 1973 to 1979. And the frequency of lysogenic strains in the Philippines were 91% (95/104) in 1973, 67% (26/39) in 1975, 85% (33/39) in 1976 and 16% (23/144) in 1978. In their report it is also assumed that the lysogenicity of vibrio would not be lost by storage of the strains *in vitro*. Comparing with our result, the rates of lysogenic strains increased to 89.8% from 16% in 1978 (Ohashi *et al.*, 1981) or 33.3% in 1984 (Ichinose, 1985).

Concerning drug sensitivity, Kuwahara *et al.* (1967) reported the drug sensitivity of about 1,500 *eltor* vibrio strains isolated in 1964 and 1965 in the Philippines. All the strains tested were highly sensitive to furalazine, and most were highly sensitive to TC, CP and EM excepting one TC-resistant (MIC: 100 mcg/ml) and three CP-resistant (MIC: >100 mcg/ml) strains, moderately sensitive to SM, KM, novobiocin and neomycin. They showed a remarkable fluctuation of sensitivity to AB-PC, cefaloridine, cefalotin and sulfafurazole, and high resistance to benzylpenicillin sodium, oleandomycin and spiramycin. Highly SM-resistant strains (MIC: >100 mcg/ml) were found in 72 out of 1,551 isolates. Subsequently, Iwanaga *et al.* (1970) observed nearly the same sensitivity pattern from 27 isolates in 1968 as Kuwahara *et al.* (1967).

Kobari *et al.* (1970) reported the drug-resistant strains in isolates from 11 out of 1,109 patients of *eltor* cholera in the Philippines. All strains were highly resistant to SM (MIC: >100 mcg/ml) and CP (MIC: 50 or 100 mcg/ml), and a few of them were resistant to TC (MIC: 25 and 50 mcg/ml). Utsunomiya (1971) also isolated the TC-resistant strain (MIC: 50 mcg/ml) from 10 out of 684 patients from San Lazaro Hospital, Manila in 1970.

Next, Iwanaga *et al.* (1979) examined the sensitivity test for 97 strains isolated from Kenya in 1975, and 52 strains from the Philippines in 1973 to 1978. By the isolates from the Philippines, the commonest MICs were 0.2 mcg/ml for minocycline (as a substitute of TC), 0.39 mcg/ml for NA, and 0.78 mcg/ml for CP. The commonest MICs of AB-PC, EM and KM were 1.56 mcg/ml to 12.5 mcg/ml. On the contrary, CER and SM showed higher resistant tendency compared with other drugs, but drug resistant strains were not detected.

On the other hand, TC-resistant *V. cholerae* were isolated from Tanzania (Towner *et al.*, 1979, 1980), Bangladesh (Glass *et al.*, 1980) and Kenya (Ichinose, 1985). In the case of the Philippines, TC-resistant *V. cholerae* were isolated in 1964 (Kuwahara *et al.*, 1967), in 1969 (Kobari *et al.*, 1970) and in 1970 (Utsunomiya, 1971). Nevertheless, increase of TC-resistant strains was not seen after 1970 in the Philippines.

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1985年フィリピン分離コレラ菌の性状について

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1985年2月－8月にフィリピン熱帯医学研究所，及びサン・ラサロ病院において，入院コレラ患者から分離された42株と18株，計60株の *Vibrio cholerae* の性状について検査を行った．血清型では，58株が小川型，1株が稲葉型で，小川型が優勢であり残る1株は NAG ビブリオであった．ポリミキシン B，ファージ IV に対しては NAG も含めすべて耐性，ニワトリ赤血球凝集反応，VP 反応はすべて陽性であり，ヒツジ赤血球溶血能試験は培養時間によって異なった成績ながらも溶血能を証明でき，すべてをエルトール型菌と判定した．プロファージ型の決定では，κ ファージの検出，及び同感受性の試験を行い，セレベス原型が89％と主体を占め，残りは cured type であり，古典ウボール型はなかった．vibriostatic agent である O/129 に対してはすべての株が感受性を示した．近年，タンザニア・ケニアなどアフリカや，バングラデシュで分離されるコレラ菌の TC 耐性化，及び多剤耐性化が重大な問題となってきているが，今回の薬剤感受性試験では，TC には高感受性を保有しており，また多剤耐性株も検出されなかった．

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