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The Vibrio Cholerae Isolated During Cholera Epidemics in Korea in 1969 and 1970.

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ABSTRACT

Recently, two successive epidemics of cholera were observed in Korea. The first one started in Suhchun-Goon of Choong-Chung-Namdo in August 1969, and the 2nd in Changyoung-Goon of Kyung Sang-Namdo in August 1970. With stool specimens collected from patients in Suhchun, Ko-Chang, Seoul, Inchun, Youngkwang, Chang-hang and Wooljin were epidemic areas in 1969, and from patients in Chang-Young, Pusan, Taegu, and Seoul which were epidemic areas in 1970, studies were carried out in 1) the isolation and identification of cholera vibrio strains 2) the differentiation of El Tor vibrio from classic cholera strains 3) the liberation test of Kappa-type phages and 4) El Tor phage typing. Five strains, which were isolated in the epidemic area of the Philippines in 1969 were included for a comparative study. The results are summarized as follows.

1) The epidemic strains of 1969 were identified as Vibrio cholerae, Celebes type El Tor and those of 1970 epidemic as Vibrio cholerae, biotype El Tor, El Tor phage type IV.

2) Korean strains and Philippine strains of 1969 epidemic appeared to be identical in biochemical and serological tests and phage susceptibility tests.

INTRODUCTION

Cholera has never been considered endemic in Korea,and therefore, introduction of the causative agent from without through various routes resulted in the development of the epidemics.

Historically, the first of the recorded epidemics of cholera in Korea occurred in the 15th Century during the Lee Dynesty (Chun, 1965), and since then, more than 30 epidemics have struck the nation. Classic cholera vibrio have been incriminated as epidemic strains in those epidemics before the occurrence of El Tor cholera epidemics recently. However, since 1960 large epidemics of cholera by El Tor vibrio, which was isolated for the first time at El Tor Quarantine station in Sinae (Gotschlich, 19 05, 1906), have been observed in Southeast Asian countries, and we experienced the first cholera epidemic by El Tor vibrio in 1963 and again in 1964.

In 1969, the first cases of cholera occurred in Choongnam Province in August and resulted in a total number fo 1583 patients

throughout the country. A small epidemic of cholera was observed consecutively in 19 70, of which the first cases occurred in Kyungnam province in August.

In this communication, a summary of cumulative data on the epidemic strains of 1969 and 1970 cholera epidemics will be reported in regards to 1) identification of the epidemic strains, 2) the differentiation of El Tor vibrios from classic cholera vibrios, 3) the liberation test of Kappa-type phage, and 4) El Tor phage typing.

MATERIALS AND METHODS

1. Isolation and identification of epidemic strains.

The specimens collected from patients by rectal swab were inoculated in to alkaline peptone water (pH 8.5) and cultivated at 37°C for 6-8 hours, and then were transfered to TCBS agar media (Eiken Chem. Co. Japan) to be cultivated again at 37°C for 18-24 hours. After cultivation typical transparent colonies were picked. The colonies which were gram-negative bacillus and agglutinated by cholera polyvalent antiserum were transfered to nutrient agar (pH 8.5), and subcultured everyday for further use.

A total of 396 strains from 1969 epidemics, and 75 strains from 1970 epidemics were included in this study. Identity and specification of the strains isolated during 1969 and 1970 epidemics are shown in table 1. Five strains which were isolated from the Philippines epidemic were provided by the courtesy of W.H.O. These were V.

Table 1. Tested strains

		Strains	Remarks
Strains isolated in 1969 in Korea		Suhchun-strain	Yonsei Univ. College of Medicine
		Seoul-strain	N.I.H. (Korea)
		Kochang-strain	〃
		Inchon-strain	〃
		Yungkwang-strain	〃
		Changhang-strain	〃
		Wooljeen-strain	〃
Strains isolated in 1969 in Philippines		D-Malibong(Ogawa)	W.H.O.
		Ogawa-9	〃
		Ogawa-10	〃
		Ogawa-12	〃
		Ogawa-67373	〃
Strains isolated in 1970 in Korea		Pusan-strain	Kyungnam Hygienic Laboratory
		Changnyong-strain	Pusan Nat. Univ. College of Med.
		Taegu-strain	Kyungpook Nat. Univ. College of Med.
		Seoul-strain	Seoul City Hygienic Lab.
Control	Strains isolated 1963 in Korea (El Tor Cholera)	V.cholerae, Ogawa 63008	N.I.H. (Korea)
		V.cholerae, Ogawa 63502	〃
		V.cholerae, Ogawa 63906	〃
		V.cholerae, Ogawa 63003	〃
	Classic vibrio cholera	V.cholerae, Inaba 35-A-3	〃
		V.cholerae, Ogawa 41	〃

cholerae, serotype Ogawa, 2 strains of serotype Inaba and Ogawa of classic cholera, and 4 strains of V.cholerae El Tor which were isolated and identified during the cholera epidemic in Korea in 1963 were included as control strains.

Halophilism, pellicle formation, cholera red test, biochemical reaction of Heiberg group I, gelatin liquefaction, and dulcitol fermentation test were carried out for further identification.

2. The differentiation of El Tor from classic cholera strains.

To differentiate El Tor from classic cholera strains, the following tests were carried out: (1) Voges Proskauer reaction (W.H.O., 1966) (2) Hemolysis test (Feeley, 1963) (3) Sensitivity to Mukerjee phage type Ⅳ (Mukerjee, 1963, 1965) (4) Polymyxin B test (1966) (5) Trypsin test (Gan, 1963) (6) Hexamine test (Ko, 1967) (7) Chicken red blood cell agglutination test (Finkelstien, 1963).

3. The liberation test of Kappa-type phage.

Kappa-type phage and the Host Vibrio cholerae H218 Smr were kindly provided by Prof. Takeya of Kyu-Shu Univ., Japan. These were tested by the methods of Takeya (1967).

4. El Tor phage typing.

El Tor phage was typed by the method of Mukerjee (1957, 1959, 1970) with phage group Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅴ and host strain (Makassar 757) was obtained from NIH Korea.

5. Electron microscopic observation.

The strains which were isolated during the epidemic in Korea in 1969 and 1970, were identified and observed by electron microscope in the Electron Microscope Laboratory of Yonsei University. These were observed by the shadow-casting method.

RESULTS

1. The isolation and identification of Cholera Vibrio strains:

Table 2. Identification of V. cholerae

Biochemical characterisitics \ Strain	Strains isolated in 1969 in Korea	Strains isolated in 19 69 in Philippine	Strains isolated in 1970 Korea	Control	
				El Tor vibrio	Classic vibrio
Colony on TCBS medium	T*	T	T	T	T
Gram's stain	G**	G	G	G	G
Motility	+	+	+	+	+
Slide agglutination with Ogawa & Inaba antiserum	+++ (Ogawa & Hikojima)	+++ (Ogawa & Inaba)	+++ (Ogawa)	+++ (Ogawa & Inaba)	+++ (Ogawa & Inaba)
Halophilism					
0% NaCl	+	+	+	+	+
3% NaCl	+	+	+	+	+
5% NaCl	−	−	+	−	−
Pellicle formation	+	+	+	+	+
Cholera red reaction	+	+	+	+	+
Biochemical reaction of Heiberg group I	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+
Dulcitol	−	−	−	−	−

*: Typical Transparent colony.
**: Gram negative comma shaped bacilli.

Table 3. Differentiation between El Tor Classic V. Cholerae

Biochemical characteristics \ Strains	Strains Isolated in 1969 in Korea	Strains isolated in 1969 in Philippines	Strains isolated in 1970 in Korea	Control	
				El Tor vibrio	Classic vibrio
Voges Proskauer reaction	+	+	+	+	−
Chicken red blood cell agglutination	+	+	+	+	−
Hexamine test	+	+	+	+	−
Trypsin test	−	−	−	−	+
Hemolysis test					
Hemolysis	50	15	18	2	2
non-hemolysis	320	0	31	0	0
Polymyxin B test					
50u/ml	R	R	R	R	S
100u/ml	〃	〃	〃	〃	〃
500u/ml	〃	〃	〃	〃	〃
Sensitivity to Mukerjee phage type IV	R	R	R	R	S

R: Resistant. S: Sensitive.

Table 4. Detection of Kappa-type phage

Strains		No. of tested strains	Release of Kappa-type phage	
			No. of Positive	No. of Negative
Strains isolated in 1969 in Korea		32	31	1
Strains isolated in 1969 in Philippines		5	5	0
Control	El Tor vibrio	2	2	0
	Classic vibrio	2	0	2

The results of identification of V. cholerae with twenty-six strains from the cases in 1969, and twenty strains from the cases in 1970 are shown in Table 2. The shape of the colonies were typical transparent cholera colonies on TCBS media. All strains were the Ogawa type and agglutinated in Ogawa antiserum except one Hikojima strain (strain of 1969 epidemic) and one Inaba strain (strain of 1970 epidemic). Table 2 shows the results of the motility, pellicle formation, cholera red test, the biochemical reactions of Heiberg group I, gelatin liquefaction and the dulcitol fermentation. It shows that each strain of the epidemic of 1969, and of 1970 were typical cholera vibrio.

2. The differentiation of El Tor from Classic cholera strains:

Table 3 shows the results of V-P reaction, hemolysis sensitivity to Mukerjee phage Ⅳ, polymyxin B test, trypsin test, hexamine test, and the chicken red blood cell agglutination test. Each strain of both 1969 and 1970 were El Tor Vibrio cholerae.

3. The liberation test of Kappa-type phages:

The liberation test of Kappa-type phage was carried out to decide if it was Celebes type or Ubon type. Out of 31 strains of the 1969 epidemic, 31 strains liberate Kappa-type phage, and one strain showed lysis by Kappa-type phage (Table 4). This result

Fig. 1. V. cholerae isolated in 1969.

indicates that the strains are Celebes type El Tor.

4. El Tor phage typing:

Twenty strains of 1970 epidemic were used to type El Tor phage. The strains showed susceptibility to group Ⅰ, Ⅱ, Ⅴ and resistant to group Ⅲ, Ⅳ. The strains belong to phage type Ⅳ as a result of the test.

5. EM studies:

Fig. 1 is the electron microscopic observation of the strains. The strains isolated in 1969 and 1970 are unipilar comma shaped bacilli which have the shape of typical cholera vibrio. This confirms the results of the former test.

6. Biochemical and biological tests of the strains which were isolated during the Phillippine epidemic 1969:

The results of identification and differentiation tests of El Tor from classic cholera strains, and liberation test of Kappa-type phage are shown in Table 2, 3 and 4. The data clearly indicate that the strains of 1969 cholera epidemics in Korea and in the Philippine were identical.

DISCUSSION

The strains of the 1969 epidemic are considered to have the same characteristics as those of the 1970's. Because this leads to the problem of cholera endemicity in Korea, it needs further investigation. The strains of the 1969 epidemic in the Philippines had the same characteristics as the strains of the 1969 epidemic in Korea, which provided bacteriological proof for the results of Lew, et al. (1969).

The strains of the epidemic in East Asia in 1969 were classified as Celebes type El Tor by Yen (1970) and by Ai (1970). There was no epidemic caused by the Ubon type. Especially the strains which were isolated from the major pandemic area (Saigon, Singapore, and Hong Kong) were known as serotype Inaba.

The strains of the 1969 epidemic in Korea were classified as Celebes type El Tor by the Cholera Research Center in India. This

indicated the possibility that the route of invasion into Korea was from the Philippines. Out of 370 strains which isolated during the 1969 epidemic, 320 strains were non-hemolytic and the remaining 50 strains were hemolytic. Out of 49 strains of the 1970 epidemic, 31 strains were non-hemolytic and 18 strains were hemolytic.

The control strains of Vibrio cholerae (Classic cholera), Inaba 35-A-3 and Ogawa 41, were also hemolytic.

Wahba (1962), Vassiliadës (1965), and Del Favers and his colleagues(1938) reported that even non-hemolytic Vibrio cholerae showed hemolysis if it was subcultured several times in the liquid culture or a solid culture which contained glucose.

Bernard Guillerm and Gallut (1939) reported that El Tor Vibrio discharged hemolysin out of the cell while classsic Vibrio contained hemolysin in the cell. This indicates that classic Vibrio also can discharge hemolysin if it meets the condition. Doorenbos (1932) caused lysis of the classic cholera with bacteriophage and observed that classic vibrio discharged hemolysin. This indicates that the hemolysis test is not a reliable method for the differentiation of El Tor from classic cholera.

Park (1969) reported that the strains of the 1969 epidemic showed susceptibility to polymyxin B. But this is different from the results of the tests which were done by the authors. Because of these different results, it was tested by the mothods of log dilution of polymyxin B to confirm the results. Two strains of El Tor cholera, and 16 strains isolated from patients were tested in the culture containing polymxin B (Pfizer Co.) which was diluted to a certain degree. Two strains of classic cholera could grow in 3 gamma and only one strain could grow in 3.5 gamma and neither of the 2 classic cholera strains could grow in more than 4 gamma.

Two strains of El Tor cholera (control strains) and 16 other strains could grow in 100 gamma, and one strain could grow even in 1000 gamma. This data confirmed the results of the authors and showed that the polymyxin B resistance test was a reliable method for the differentiation of El Tor cholera from classic cholera.

The polymyxin B resistance tests of the 1969 and 1970 epidemic strains by Chun (1970) showed the same results as the authors. Because the result of the Mukejee phage Ⅳ sensitivity test was not in agreement with that of Lee (1969), it was tested again. The strains were classified as El Tor cholera which agree with Chun's(1970) view. It was reported that the Mukerjee phage Ⅳ sensitivity test was accurate in sensitivity and in specificity (Mukerjee 1963, 1965). Therefore it is clear that the epidemic strains were El Tor cholera.

All of the 32 strains of the 1969 epidemic were lysed in Kappa-phage except one. This indicates that the one strain which was not liberated is also sensitive to Kappa-type phage. In view of Chun's (1970) conclusion that the strains of the 1970 epidemic liberated the Kappa-type phage, the strains of 1969 and 1970 might liberate the Kappa-type phage and also Celebes type phage.

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