Types and Drug Sensitivity of Bacterial Enteropathogens Isolated in Neighboring Countries of Japan

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Abstract

Phage type M1 was most frequently found(44%) among 190 *Salmonella typhi* strains isolated from sporadic cases in South Korea. An epidemic of typhoid has broken out in a province, 60 isolates from which were typed in all as type A. Phage types of *S. typhi* in Taiwan was extremely rich in variety, among them some rare phage types like G, E9 and T were found. There was no appreciable difference in drug sensitivity between *S. typhi* isolated in Korea and Taiwan on one hand, and between the strains from sporadic and epidemic cases in Korea on the other hand. The results of serotype determination, biochemical study and drug sensitivity tests in 78 salmonellae other than *S. typhi* are also presented. Sixty strains among them were of human source, and were forwarded to the laboratory for identification from Korea, Taiwan, the Philippines, and some public health institutions in Nagasaki. The remaining 18 strains were those isolated from laboratory animals in Taiwan. It is noteworthy that *S. paratyphi C* was found only in Korea, *S. typhimurium* was more frequent in the other countries, and, in the Philippines, two multiply resistant strains showing resistance extending over kanamycin, aminobenzil-penicillin and fradiomycin were isolated. In addition, it was observed that all salmonellae and shigellae used, numbering 363 and 47 respectively, showed a high sensitivity to furazolidone and furatrizine, especially to the latter. The most prevalent colicin type of *Shigella sonnei* in Taiwan was type 8, whereas in Hong Kong, type 9A. Distinct differences with regard to biochemical type and drug-resistance pattern of this organism were not observed between the two countries. Out of a total of 56 halophilic bacteria, isolated from sea-water and fishes in Taiwan, only one strain was determined as sucrose negative, hemolytic and K-typable *Vibrio parahaemolyticus*.

Special Contribution

Southeast Asia is known as an area where enteric diseases constitute a major preventative-medical problem. Japan and South Korea, locating in the temperate zone, also are similarly ciscumstanced ; in Japan dysentery due to *Shigella sonnei* and bacterial food poisoning are still prevalent, and in South Korea there is a tendency to increase in typhoid fever.

It is a good problem for us to study the cause of enteric infections including bacterial food poisoning in every Asiatic country. In this regard, it is worthy to note that in Japan causing agents of bacterial food poisoning have been cleared up in about 60 percent of the cases since *Vibrio parahaemolyticus* was discovered and its pathogenicity established.

One of the main studies of this Department is to reveal the actual state of type distribution of *Salmonella* and *Shigella* in Asiatic countries,[4)5)6)9)10)14)] and the other is to search for *V. parahaemolyticus* in food poisoning cases in foreign countries and in marine products there.[7)11)] In order to obtain some additional material for the accomplishment of the both aims, the author collected strains of the mentioned bacterial groups, once identified or unidentified, from Korea, Taiwan, the Philippines, and Hong Kong during the period mainly from 1966 to 1968, and made a study of their serotypes, sensitivity to antibiotics and chemotherapeutic drugs, phage type particularly in *S. typhi*, and colicin type as well as biochemical type in *Sh. sonnei*, etc.

Material and Methods

Cultures examined : A total of 463 cultures consisting of 363 salmonellae, 47 shigellae and 56 halophile bacteria were used for the present studies. These strains, exculsive of 32 *Salmonella* strains isolated in Nagasaki and Sasebo City, were obtained from the following foreign donors : Dr. Doki Chun, Department of Bacteriology, Kyung-Pook University Medical School, Taegu, Korea ; Dr. Inn Soo Suh, Department of Microbiology, Woo Sok University Medical College, Seoul, Korea ; Dr. Shu-Tao Hsu, Taiwan Serum Vaccine Laboratory, Taipei, Taiwan, China ; Dr. Ching-Mu Cheng, Department of Veterinary Medicine, Taiwan Provincial Institute of Agriculture, Pingtung, Taiwan, China ; Dr. Cecilia Z. Gomez, Department of Health, Bureau of Research and Laboratories, Manila, the Philippines ; and Dr. C. T. Huang, Department of Bacteriology, University of Hong Kong, Hong Kong.

The first group of salmonellae consisted of 29 strains isolated in Korea in 1957 and maintained in this Department till now, and of 236 strains isolated there during the years 1966-1968. They were wild strains isolated mainly from typhoid and paratyphoid patients. The author was informed that there were some strains originated from carriers but no strains from patients being symptomatic of typical food poisoning. The regional distribution of this group, totaling 265, was as follows : Seoul (19), Pusan (12), Kyonggi (3), Kangwon (60), Chung-Nam (2), Chon-Nam (1), Kyung-Pook (105),

Kyung-Nam (16), and 47 strains of unknown isolation place. All Kangwon strains, among them, were said to have been isolated from a mass outbreak.

A second group of 52 salmonellae received from Taiwan Serum Vaccine Laboratory consisted of 25 strains of *S. typhi*, two strains of *S.paratyphi A*, and 25 unidentified strains. Eighteen out of 25 unidentified strains were isolated from epizootics among laboratory animals (14 guinea-pigs and two mice) occurred several times in the mentioned Laboratory, and from two snakes which made a forcible entry into a pen for small animals there. The other cultures, together with 25 once identified strains of *S.typhi* and two *S.paratyphi A*, were of human origin and had been submitted to the Laboratory from the Taipei area, mainly from Provincial Keelung Hospital (the infectious disease hospital).

The remaining 14 and 32 salmonellae are grouped into the third and the fourth classes, respectively. The former came from Manila, the Philippines, meeting the author's demand for salmonellae other than *S.typhi*. The latter were *Salmonella* strains forwarded to this Department from Nagasaki and Sasebo Health Center for identification between September 1966 and February 1968. Among them, there were seven strains isolated from foreign residents in Sasebo City and from foreigners who had just returned there from the south.

As for collection of shigellae the main stress was laid on *Sh.sonnei*, and, indeed, 39 out of 47 forwarded strains were identified with this organism. The collection of *V.parahaemolyticus* was made only from Taiwan, and 56 strains of it, namely three strains isolated from sea water of Keelung Port and 53 strains from marketing fishes and shellfishes in Keelung, Kaohsing and Pingtung, were submitted to examination. No strain could be detected from material of human origin.

Identification : Cultures were identified in accordance with cultural and serologic methods commonly employed, particularly those described by Edwards and Ewing (1962)[17] and by Kauffmann (1966)[24] in the case of *Salmonella* and *Shigella*, and by the standarized method for *V.parahaemolyticus* recommended publicly in the name of the Ministry of Welfare in 1961. Diagnostic antisera used were for the most part Toshiba's products, consisting of polyvalent sera for a rough classification and of factor or monovalent sera for determination of antigenic structure (*Salmonella* and *Shigella*) or for K-typing in *V.parahaemolyticus*. In addition, some *Salmonella* factor sera of the author's own preparing were used as occasion demanded. Rare serotypes and doubtful strains were sent to Dr. R. Sakazaki, Japan *Salmonella* Center, Tokyo, or Dr. H. Zenyoji, Tokyo-to Laboratories for Medical Sciences, for final identification or confirmation.

Determination of microbial sensitivity : This tests were made only with strains of salmonellae and shigellae. As recommended by the Research Committee of Enteric Group Bacteria,[18][39] the plate dilution method was employed. Media—— Peptone water (pH 7.2) for enrichment, and heart infusion agar "Eiken" for the

seed layer in which each antibiotic or drug (drugs in short) was incorporated. Drug dilutions——Powder of streptomycin (SM), tetracycline(TC), chloramphenicol (CP), erythromycin(EM), kanamycin(KM), aminobenzil-penicillin (AP), polymyxin B (PB), fradiomycin (FM), furazolidone (FZ), and furatrizine (FT) were used for the sensitivity tests, of which 1000 mcg/ml aqueous solutions were prepared. Inoculation and incubation —— After inoculation in peptone water for 18-24 hours, each strain was streaked on the heart infusion agar containing each of the drugs in various concentrations. In order to prepare this series of media, two-fold serial dilutions of the original drug solution were made up to give the final concentration of 3.90 mcg/ml, and each dilution was added to the medium at a ratio of 1 to 9. Reading the results —— The results were read as + for growth as in controls, ± for small or fewer colonies, and − for no growth, and results were expressed in terms of the minimum growth-inhibiting concentration of each drug. In every case, a control *Salmonella* strain(*S. typhimurium* No. 670308, Taiwan) or a *Shigella* strain (*Sh. flexneri* 1b, EW-9) having known sensitivities was tested for growth on media with and without drugs to ensure uniform results.

Phage typing of *S. typhi* : The phage typing was carried out through the courtesy of the National Laboratory for Enteric Phage Typing located in the National Institute of Health, Tokyo. Typing phages used as well as typing technique can be seen in a report of Fukumi et al.[19] in this Laboratory.

Colicin typing and tests for biochemical type : The both experiments were performed only on *Sh. sonnei*. Colicin types were determined following the method of Abbott and Shannon,[1] and of Naito.[27] Full particulars of the technique have been described in some reports presented by this Department.[8][12]

Results

The *Salmonella* serotypes identified are shown in Table 1. Two hundred and fifty out of 265 *Salmonella* strains, or 95 percent, isolated in Korea, were identified with *S. typhi*, and the remaining 15 strains were as follows : 8 *S. paratyphi B*, 3 *S.paratyphi C*, 2 *S.enteritidis*, and each one *S.paratyphi A* and *S. typhimurium*. Except for 47 strains of unknown origin, the isolation place of the strains converges for the most part on Seoul and its suburbs(22), Kangwon-Do (60), Kyung-Pook-Do (105), and Kyung-Nam-Do including Pusan (28). Among them, only the strains from Kangwon-Do had been reported to be isolates from a mass outbreak of typhoid fever in a collective body, and that this state of affairs was well supported not only by identification of all the isolates with *S. typhi* possessing Vi-antigen but also by following results of phage typing.

Source of 4 out of 8 strains of *S.*

Table 1. *Salmonella* serotypes identified

Region	Year	No. of strains	Origin	Serotypes
Korea	1957	29	Human	S. typhi : 28 S. paratyphi B : 1
	1966–68	236	Human	S. typhi : 222(60) S. paratyphi B : 7 S. paratyphi C : 3 S. enteritidis : 2 S. paratyphi A : 1 S. typhimurium : 1
Taiwan	1964	12	Human	S. typhi : 12
	1967	22	Human	S. typhi : 13 S. typhimurium : 3 S. derby : 3 S. paratyphi A : 2 unidentified : 1
	1964–67	18	Animal	S. newport : 9 S. typhimurium : 6 S. weltevreden : 2 S. schwarzengrund : 1
Philippines	1964–66	14	Human	S. typhimurium : 6 S. newport : 2 S. worthington : 2 S. potsdam : 1 S. javiana : 1 S. taksony : 1 unidentified : 1
Nagasaki	1962–66	25	Human (Japanese)	S. typhi : 10 S. typhimurium : 12(12) S. enteritidis : 1 S. bredeney : 1 unidentified : 1
Sasebo	1966, 67	7	Human (Foreigner)	S. anatum : 3(3) S. montevideo : 3(2) S. heidelberg : 1

The parenthesized numbers indicate those of strains isolated from mass outbreak cases or cases attributable to contact infection in an institution or in a family, forming a part of the figures listed.

paratyphi B, 2 out of 3 Vi-positive *S. paratyphi C* strains as well, has been not made known beyond that they are originated from sporadic cases, but informed that the remaining 4 *S.paratyphi B* strains, together with the other 5 *Salmonella* strains other than *S.typhi*, are those isolated from sporadic cases among civilian. Isolation places and serotypes of them were : Kyung-Pook—— 2 *S.paratyphi B*, each one *S.paratyphi A*, *S.paratyphi C*, *S.typhimurium* and *S. enteritidis* ; Seoul —— each one *S. paratyphi B* and *S.enteritidis* ; Pusan —— one *S.paratyphi B*.

Salmonellae other than *S.typhi* isolated in the other countries showed a variety in serotype. *S. typhimurium*, totaling 27 strains, ranked first in the total amount, but it must be taken into consideration that there were six strains isolated from laboratory animals in Taiwan on one hand, and 12 strains from each case of food poisoning occurred in a mental hospital in Nagasaki City on the other hand. As the other strains of human origin were in the minority each, the author can not make them meaningful for comparison of type distribution in the respective countries, but it is noteworthy that *S.paratyphi C* was isolated only in Korea. There are two not yet identified strains belonging to El group, which showed following reactions to five diagnostic sera used :

	Taiwan 670310	Philip. 66188
E (3,10,19)	+	+
El (10)	+	+
e,h (h)	−	+
r (r)	+	−
1 (1,2,5,z_6)	+	+

With 11 *S.typhi* and all *Salmonella* strains other than *S.typhi*, five biochemical reactions necessary for differential diagnosis between the four sub-genera of Kauffmann[24] and reactions for other properties listed in "blochemical reactions" of the Kauffmann-White-Schema(1966)[24] were made. They gave all a positive dulcitol test only, and so they were diagnosed all as strains of sub-genus I. As for the other reactions, for examples sodium citrate in *S.typhi* and d-tartrate in *S.typhimurium*, there were some strains showing no agreement with those listed in the Kauffmann's table.

In the case of *S.typhi*, after performing tests for biochemical and serological identification with it and the test for Vi-antigen, the author entrusted the National Laboratory for Enteric Phage Typing in Tokyo with Vi-phage typing. The number of *S.typhi* listed in Table 1, totaling 285, decreased to 262 following the screening by Vi-agglutination. The results of phage typing reported from the National Laboratory are shown in a classified table by regional groups(Table 2).

It has been shown in this table that (1) phage type M1 is most frequently found among strains isolated from sporadic cases in Korea ; (2) the typhoid epidemic in Kangwon-Do was caused without exception by the type A ; and (3) phage types of strains isolated in Taiwan are extremely rich in variety. There is no telling about typing results of strains isolated in Japan, because they are too small in number to permit exact comparison with the other data. It deserves brief mention, in addition, that there were each one strain of type G and type K1 among the strains from Korea and Taiwan respectively, nonexistence of which in Japan have been shown in three reports[19][29][30] presented by the National Laboratory.

Table 2. Phage types of *S.typhi* by regional groups

Phage types	Region, Origin and No. of strains			Total
	Korea	Taiwan	Japan	
A	70(60)	4	2	76(60)
B 2		1		1
D 1		2		2
D 2	3	4	3	10
D 6	3			3
E 1	1	2	1	4
E 4	2			2
E 9		1		1
G		1		1
K 1	1			1
L 1		2		2
M 1	83	3		86
T		2		2
46	2		1	3
A degraded	59	1		60
untypable	5	1	2	8
Vi-negative	21(0)	2		23(0)
Total	250(60)	26	9	285(60)

The numbers in parentheses indicatet he finding on a mass outbreak case.

All the strains of salmonellae were tested for sensitivity to ten kinds of antibiotics and chemotherapeutic drugs. Results obtained are indicated separately in Tables 3 and 4, being divided into two bacterial groups and arranged further

Table 3. Drug sensitivity of *S.typhi*, arranged according to country

Area (No. of str.)	Drugs	Minimum inhibitory concentration (mcg/ml)									
		>100	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39*
Korea (250)	SM	2	22	111	76	27	8	0	0	4	0
	TC	0	0	0	0	0	1	5	121	122	1
	CP	0	0	0	0	30	65	141	13	1	0
	EM	14	64	131	39	2	0	0	0	0	0
	KM	0	0	0	0	0	2	47	196	5	0
	AP	0	0	0	0	0	1	14	58	47	130
	PB	0	0	0	9	98	109	32	2	0	0
	FM	0	0	0	0	0	0	1	70	131	48
	FZ	0	0	0	0	0	0	0	34	162	54
	FT	0	0	0	0	0	0	0	1	73	176
Taiwan (25)	SM	1	13	7	4	0	0	0	0	0	0
	TC	0	0	0	0	0	0	0	3	22	0
	CP	0	0	0	0	0	0	25	0	0	0
	EM	0	18	7	0	0	0	0	0	0	0
	KM	0	0	0	0	0	0	3	21	1	0
	AP	0	0	0	0	0	0	0	2	6	17
	PB	0	0	1	16	6	2	0	0	0	0
	FM	0	0	0	0	0	0	0	1	5	19
	FZ	0	0	0	0	0	0	0	5	0	20
	FT	0	0	0	0	0	0	0	0	0	25
Japan (10)	SM	0	1	4	4	1	0	0	0	0	0
	TC	0	0	0	0	0	0	0	2	7	1
	CP	0	0	0	0	0	0	0	3	3	4
	EM	0	1	6	1	0	2	0	0	0	0
	KM	0	0	0	0	0	0	2	5	3	0
	AP	0	0	0	0	0	0	0	1	2	7
	PB	0	0	0	0	6	2	2	0	0	0
	FM	0	0	0	0	0	0	0	0	3	7
	FZ	0	0	0	0	0	0	0	0	2	8
	FT	0	0	0	0	0	0	0	0	2	8

* including a concentration less than 0.39 mcg/ml.

according to country.

There was little difference in drug sensitivity between *S.typhi* isolated in Korea and Taiwan(because of a minority of strains used, the data on Japanese strains are not available for the present purpose). The same could be said of the two subgroups of Korean strains, namely strains isolated from sporadic cases and those from the typhoid epidemic occurred in Kangwon-Do. Generally speaking, with SM and EM the strains were inhibited for the most part by concentrations of 12.5 mcg/ml or more, with PB by concentrations covering the range of 3.13-25 mcg/ml, with CP and KM in the range of 1.56-12.5mcg/ml, and with the other drugs by concentrations of

Table 4. Drug sensitivity of salmonellae other than *S.typhi*

Area (No. of str.)	Drugs	Menimum inhibitory concentration (mcg/ml)									
		>100	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39*
Korea (15)	SM	0	2	1	2	6	3	1	0	0	0
	TC	0	0	0	0	0	0	1	10	3	1
	CP	0	0	0	0	0	3	9	2	1	0
	EM	0	3	8	1	1	2	0	0	0	0
	KM	0	0	0	0	0	3	3	7	2	0
	AP	0	0	0	0	0	0	0	10	4	1
	PB	0	0	0	4	11	0	0	0	0	0
	FM	0	0	0	0	0	0	1	3	8	3
	FZ	0	0	0	0	0	0	0	6	6	3
	FT	0	0	0	0	0	0	0	0	1	14
Taiwan (27)	SM	1	2	4	10	6	0	3	1	0	0
	TC	0	0	0	0	0	5	3	19	0	0
	CP	0	0	0	0	1	18	6	2	0	0
	EM	3	17	6	1	0	0	0	0	0	0
	KM	0	0	0	0	1	3	14	8	1	0
	AP	0	0	0	0	0	1	5	21	0	0
	PB	0	0	2	12	10	3	0	0	0	0
	FM	0	0	0	0	0	0	1	9	13	4
	FZ	0	0	0	1	0	0	4	16	1	5
	FT	0	0	0	0	0	0	0	1	2	24
Philippines (14)	SM	4	1	3	3	3	0	0	0	0	0
	TC	3	0	0	0	0	1	3	7	0	0
	CP	2	0	0	0	0	6	6	0	0	0
	EM	0	11	3	0	0	0	0	0	0	0
	KM	2	0	1	0	0	8	3	0	0	0
	AP	2	0	0	0	0	0	0	11	1	0
	PB	0	0	0	0	12	2	0	0	0	0
	FM	2	0	0	0	0	1	0	10	1	0
	FZ	0	0	0	0	0	0	6	7	1	0
	FT	0	0	0	0	0	0	0	0	8	6
Japan (22)	SM	8	6	5	3	0	0	0	0	0	0
	TC	2	0	0	0	0	0	7	12	0	1
	CP	0	0	0	0	0	19	3	0	0	0
	EM	1	21	0	0	0	0	0	0	0	0
	KM	0	0	0	0	2	11	9	0	0	0
	AP	0	0	0	0	0	0	6	16	0	0
	PB	0	0	3	6	13	0	0	0	0	0
	FM	0	0	0	0	0	2	2	13	4	1
	FZ	0	0	0	0	0	0	5	10	0	7
	FT	0	0	0	0	0	0	0	0	5	17

1.56 mcg/ml or less. There were 39 strains (13.7%) showing minimum inhibitory concentrations of 100 mcg/ml or more as to SM, and 97 strains (34.0%) as to EM among 285 *S.typhi* strains tested. The acquired resistance of a high level to SM and EM is considered to be independent each other ; only 22 strains held them in common.

Salmonellae other than *S.typhi* presented an almost complete similarity in drug sensitivity to *S.typhi*, except that following four multiply resistant strains according to the definition form ulated by Japanese workers (sensitiviy in 100 mcg/ml or more) were found :

S.potsdam (Philippines 65204) SM TC CP KM AP FM

S.worthington (Philippines 65380) SM TC KM AP FM

S.worthington (Philippines 65367) SM CP

S.anatum (Sasebo 807) SM TC

Shigellae were collected, laying stress on subgroup D, namely *Sh.sonnei*. Serotypes identified of a total of 47 strains were as follows : Korea——each one *Sh.flexneri 1a, 1b* and *2a*, and five *Sh.sonnei* ; Taiwan —— 20 *Sh.sonnei* ; Hong Kong —— 13 *Sh.sonnei* ; and the Philippines —— each one *Sh.dysenteriae 2*, *Sh.flexneri 1b* and *Sh.sonnei*, and two *Sh.flexneri var. Y*.

As shown in Table 5, variations in sensitivity of shigellae to the drugs were more marked than was observed in salmonellae, and, generally speaking, it can be said that sensitivity of shigellae

Table 5. Drug sensitivity of shigellae

Group	Drugs	Minimum inhibitory concentration (mcg/ml)									
		>100	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39*
Shigellae other than Sh. sonnei (8)	SM	5	0	0	0	0	0	2	1	0	0
	TC	0	0	0	1	3	0	1	2	1	0
	CP	2	0	1	0	0	0	0	3	1	1
	EM	0	1	2	2	2	1	0	0	0	0
	KM	0	1	0	0	0	4	3	0	0	0
	AP	1	0	1	0	0	2	2	1	1	0
	PB	0	0	0	0	1	0	4	3	0	0
	FM	1	0	0	0	0	1	4	2	0	0
	FZ	0	0	0	0	1	0	0	0	5	2
	FT	0	0	0	0	0	0	0	1	0	7
Sh. sonnei (39)	SM	27	1	0	1	0	1	6	1	2	0
	TC	19	2	0	0	6	0	3	5	3	1
	CP	23	0	0	0	0	1	13	1	0	1
	EM	0	2	33	3	0	0	0	1	0	0
	KM	0	0	0	0	2	21	13	3	0	0
	AP	0	0	0	0	2	32	4	1	0	0
	PB	0	0	0	0	6	13	19	0	0	1
	FM	0	0	0	0	0	0	19	16	3	1
	FZ	0	0	0	0	0	2	6	4	11	16
	FT	0	0	0	0	0	0	0	0	10	29

was some lower especially as to SM, TC and CP. For practical purposes it is convenient to designate those strains showing sensitivity less than 50 mcg/ml to the mentioned three drugs simply as "sensitive" or S and the others as "resistant" or R. In Table 6 are indicated the results of sensitivity tests in 39 *Sh. sonnei* strains by the use of the both abbreviations, together with colicin types determined by the method of Abbott and Shannon and of Naito and with biochemical types according to Gillies[20] and Szturm-Rubinsten.[34]

Table 6. Colicin type, biochemical type and drug resistance pattern of *Sh. sonnei*

Area	Colicin type		Biochemical type		Resistance
	Abbott	Naito	Gillies	S.R.*	pattern**
Korea (5)	6	B_1	RM	a	R R R (2)
	6	B_1	M	a	R S S (1)
	0	A_1	RM	a	R S S (2)
Taiwan (20)	6	B_1	RM	a	R R R (2)
	6	B_1	RM	a	R S R (1)
	6	B_1	M	a	R R R (1)
	8	C_1	RM	a	R R R (7)
	8	C_1	M	a	R R R (2)
	8	C_1	R	a	R R R (2)
	8	C_1	RM	a	S R R (2)
	8	C_1	RM	a	S S S (1)
	12	D	M	a	R S S (1)
	12	D	R	a	S S S (1)
Hong Kong (13)	6	B_1	RM	a	R S S (1)
	6	B_1	RM	a	S S S (2)
	8	C_1	RM	a	R R R (1)
	9A	uc	RM	a	R R R (1)
	9A	uc	M	a	R R R (1)
	9A	uc	RM	a	R S S (1)
	9A	uc	RM	a	S S S (5)
	0	A_1	RM	a	R S R (1)
Philippines(1)	0	A_1	RM	a	R S S (1)

* Szturm-Rubinsten

** In the order of SM, TC, CP. Numbers in parentheses indicate those of strains forming a category.

There was a marked difference in colicin type between strains isolated in Taiwan and Hong Kong. Fourteen out of 20 *Sh. sonnei* strains (70%) isolated in Taiwan were classified as colicin type 8 of Abbott and Shannon or C_1 of Naito, whereas, in sharp contrast, eight out of 13 isolates (62%) in Hong Kong as type 9A of the so-called Japanese modification of Abbott and Shannon's method.[12][13] Another finding related to the difference between the both regions was an occurrence of the triple resistant strain. There were 14 strains showing resistance pattern RRR among 20 isolates (70%) in Taiwan, whereas only three among 20 isolates (15%) in Hong Kong. Reference may here be made to isolation of following two *Sh. flexneri* strains showing uncommon resistance patterns, extending over some other antibiotics :

Sh. flexneri 1b (Philippines 65361) SM EM KM FM

Sh. flexneri 2b (Korea 3858) SM CP AP

All of the 39 *Sh. sonnei* strains were determined biochemically as type "a" (ONPG positive, rhamnose and xylose negative) according to Szturm-Rubinsten,[34] and 30 out of them (70%) as RM (xylose negative, raffinose and melibiose positive) according to Gillies.[20]

Main cultural and biochemical characteristics of halophile bacteria, totaling 56 strains, which were forwarded to our laboratory as suspected strains of *V. parahaemolyticus*, are given in Table 7.

It is indicated in this table that all the strains tested were identified with *V. parahaemolyticus* of a wide sense or

Table 7. Cultural and biochemical characteristics of vibrios

Characteristics	Results		
Growth in 0% NaCl	+ 2	± 1	−53
3% NaCl	+56		
7% NaCl	+52	± 4	
10% NaCl	+ 9	±21	−26
Indol	+56		
Voges-Proskauer	+34	± 3	−19
Tartrate utilization	+56		
Nitrate reduction	−56		
H_2S (TSI)	−56		
Gelatin liquefaction	+51	± 5	
Cytochrom oxidase	+56		
Hugh-Le fson	+56		
Fermentation of sucrose	−51	+ 5	
arabinose	+34	−22	
cellobiose (24 hr.)	−56		
Hemolysis (Kanagawa phenomenon)			
	+37	−19	

Values represent the numbers of strain exhibiting positive, doubtful, or negative growth or reaction.

of the former name. And, indeed, they could be divided into two groups, namely, 15 strains of *V. parahaemolyticus* of a strict sense which was not or hardly capable of growing in a 10 percent NaCl-medium and at the same time exhibited the negative Voges-Proskauer reaction, and 41 strains of *V. alginolyticus*.

The results of hemolysis test newly called "test for Kanagawa phenomenon" and those of slide agglutination using a set of K-sera, however, confirmed not always the evidence for separating the both species mentioned above. In 12 out of 15 *V. parahaemolyticus* and in seven out of 41 *V. alginolyticus* strains the positive Kanagawa phenomenon could not be observed within 48 hours. The results of serological typing were as follows : *V. parahaemolyticus* —— three K 28, each two K 17, 32 and "uc", and each one K 8, 15, 24, 25, 30 and 31 ; *V. alginolyticus* —— 21 "uc", ten K 32, each two K 15, 17 and 24, and each one K 3, 10, 18 and 20.

After all, only three isolates from marketing fishes remained as strains of hemolytic *V. parahaemolyticus*. One of them, however, was unclassifiable by the serological method, and the other fermented sucrose. The detection of one other strain of K-type 32 alone will come into question.

Discussion

Typhoid fever is said to be a most prevalent acute infectious disease in Korea. The case rates per 100,000 population were 10.42, 17.89 and 11.21 in the years 1962, 1963 and 1964, respectively. In those days (June 1963 – April 1964), Kim[26] detected 10 *S.typhi*, 5 *S.paratyphi A*, 2 *S. paratyphi B*, and 17 other salmonellae carriers among 1,855 entertainers in Taegu City, and recently (February–December 1968), Ha[21] 11 *S. typhi*, 3 *S. paratyphi C*, and each one *S. choleraesuis* and *S. senftenberg* carriers among 5,776 persons living in Chon-Nam-Do. Making a comparison these conditions with those in Japan and with typhoid and paratyphoid case rates recorded in the W.H.O.'s Epidemiological and Vital Statistics Report (1962–1965), in the other countries, it is known that Korea of today is exposed most seriously to the menace of typhoid and paratyphoid

among countries in question.

Although much work has been done by the workers in medical colleges of Taegu and Kwangju on salmonellae isolated in the respective area, there are comparatively few accounts in the literature of salmonellae isolated in the north part of South Korea. In this regard, the present study will serve for something in epidemiology of salmonellosis all over South Korea.

It is said that a characteristic feature in the distribution of *S. typhi* Vi-phage types in Korea is predominance of type M1.[6)21)31)] This finding has well been supported by the present study using strains isolated from sporadic cases occurred in Seoul and its suburbs, Kyung-Pook-Do, and Kyung-Nam-Do including Pusan. In addition, there was a mass outbreak of typhoid in Kangwon-Do caused without exception by the organism of phage type A which is relatively of rare occurrence in Korea. This is considered to be a good example of applying Vi-phage typing to epidemiology of typhoid. Phage types in Taiwan was extremely rich in variety, among them some rare types like G, E9 and T were found, and in Korea there was a strain of type K1 which had not yet been found in Japan. The geographical distribution of Vi-phage types in Asia is a topic for further discussion.

Seventy-six salmonellae strains other than *S.typhi* were classified into 17 serotypes (there were two finally unidentified strains of *Salmonella* E1-group, in addition). No serotype of very rare occurrence ; the identified types have been all included in "frequent *Salmonella*" or at least "not daily observed" of Kelterborn[25)]. Because of a minority of the strains used and typed, care must be exercised in drawing a conclusion on the geographical distribution, but it is noteworthy that *S. paratyphi C* was isolated only in Korea and *S. typhimurium* prevailed among the strains isolated in Taiwan and the Philippines. On this point, following references are full of suggestions.

In Korea, during the Korean war (1950—1953), *S. paratyphi C* and *S. typhimurium* were recovered at nearly the same rates (10.3 % and 10.7%), but, refering to two follow-up studies[2)] presented in the review of Chun[15)] and to the latest information of Ha[21)], it is clearly known that *S. paratyphi C* has been found more prevailingly than *S. typhimurium* from human beings after that. On the contrary, there is no information concerning human infection or carrier of *S. paratyphi C* in Japan[33)], Taiwan[14)], and the Philippines[32)], while cases due to *S. typhimurium* have frequently been reported there. The same may be said of the continental countries of East Asia. According to Huang and Lo[23)], and Hamano[22)], the isolation rate of *S.paratyphi C* is certainly high as compared with that of *S. typhimurium* in Chinese Continent.

There was no appreciable difference in drug resistance between *S. typhi* strains isolated in Korea and those in Taiwan, and by reference to data presented by Ohashi[28)] and by Anzai and Nakamura[3)], the same may be said of strains isolated in Japan as far as this organism is concerned. As to salmonel-

lae other than *S. typhi*, it must be mentioned that there were two strains exhibiting a cross resistance extending over kanamycin, aminobenzil-penicillin and fradiomycin among 20 strains tested. One strain showing a cross resistance to aminobenzil-penicillin and cephaloridine (Tanigaki and Sugiyama[35]) and two strains with resistance to aminobenzil-penicillin (Anzai and Nakamura[3]) have already been detected among human source strains in Japan.

The antibiotic resistance of 156 salmonellae (151 *S.typhi* and 5 A-group strains) and 109 shigellae (15 *Sh. dysenteriae*, 83 *S.flexneri*, 3 *Sh.boydii*, and 8 *Sh.sonnei*) strains isolated in 1963, 1964 and 1965 in Taegu area, Korea, has been reported by Chun et al.[16] Their study regarding SM, TC and CP serves as a good reference, because it was carried out by the same method as that Japanese workers in routine used. Only four strains with the triple resistance were found among 156 salmonellae (2.6%), whereas 65 out of 109 shigellae strains (60%) were of this resistance pattern.

Because of a minority of strains used, the data on shigellae presented by the author (Ikeda) are not sufficient to lead to any conclusion on the geographical difference in an acquisition state of drug-resistance, but it can only be said with certainty that the acquisition of drug-resistance in shigellae is proceeding rapidly as compared with that in salmonellae. And, by the way, it is considered to be a datum of clinical importance that all the strains used, together with salmonellae, have showed a high sensitivity to furazolidone and furatrizine, especially to the latter. The same may be said of fradiomycin (neomycin), a preparation of aminoglycoside antibiotics, but it is worth due consideration that its antibacterial action was somewhat weaker than that of the mentioned two drugs, and that there were three strains, two salmonellae and one shigella, exhibiting the resistance above 100 mcg/ml to it.

The results on *Sh. sonnei* and halophilic bacteria should be discussed from the standpoint of co-operation in this Department, and, indeed, the data on the geographical distribution of colicin type in *Sh. sonnei* and on the possibility of detecting *V. parahaemolyticus* from sea-water and fishes have been inserted and discussed in the communications of Aoki et al.[12] including the author, and of Aoki[7][11] as a representative of the co-workers.

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近隣諸国分離腸系病原菌の型と薬剤感受性

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摘　　要

韓国の散発例分離チフス菌190株のうちではファージ型M 1が最も多かった．同国で腸チフスの集団発生があったが，その分離菌のファージ型はすべてA型であった．台湾分離チフス菌のファージ型は極めて多彩で，そのなかにはG，E9，Tのような稀有型もみられた．韓国と台湾分離チフス菌の薬剤感受性には特記すべき差異

がなく，韓国の散発株と集発株を比較しても同様であった．チフス菌以外のサルモネラ 78株の血清型決定，生化学的研究，薬剤感受性試験の成績も提示されている．そのうち 60株は韓国，台湾とフィリピンからおよび長崎県下の保健所などから送られてきた人系株，残る18株は台湾で実験動物から分離されたものであった． パラチフスC菌が韓国だけで見出されたこと， ***S. typhimurium*** はその他の国で最もしばしばみられたこと， それとフィリピンで， カナマイシン，アミノベンチールペニシリンからフラジオマイシンにも及ぶ多剤耐性菌２株が検出されたことは特記の要がある．これに加えて，使用したサルモネラ363株と赤痢菌47株はフラゾリドンとフラトリジンに対し，特に後者に対して高い感受性を示した． 赤痢ソンネ菌のコリシン型として台湾で最も多かったのは８型，香港では９A型であった． 同菌の生化学型と薬剤耐性パターンについては両地間に特に明確な差異を認めなかった． 台湾で海水と魚類から分離した好塩菌は56株，***V. parahaemolyticus*** としては15株であったが，このうち白糖非分解，溶血性，K型別可能のものは僅か１株にすぎなかった．