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Colicin Type, Biochemical Type and Drug-resistance Pattern of Shigella sonnei Isolated in Japan and Its Neighboring Countries ; A Detailed Report

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Abstract

A modification of Abbott and Shannon's colicin typing method for *Shigella sonnei* which was established recently as a standard method in Japan was described. This method increases by three or four indicators of *E. coli* K-12 mutant origin, and it is not only to make a clear distinction between either types 6 and 11 or types 4 and 14, but also to establish three new colicin types 4A, 9A and 13A. Typing results of 1,148 strains representing "foci" which were isolated in Japan especially in the western part, were presented. In Japan at present, type 14 is at the top of epidemic strains, type 6 ranks next, and types O, 8, 13A, 4, 2, etc. follow in order, among them only types 8 and 13A show some difference in interregional distribution. Besides, 39 strains isolated in the neighboring countries of Japan were used for typing. There was a most distinct difference in colicin type between here and there. The strains examined biochemically were determined by 78 percent as type RM of Gillies (xylose negative, raffinose and melibiose positive), and by 96 percent as type "a" of Szturm-Rubinsten (ONPG positive, rhamnose and xylose negative). Both the biochemical types were equally distributed among colicin types other than type 12 ; in colicin type 12, strains showing various fermentation patterns were found. The resistance of the strains to sulfa-drug and three antibiotics raised gradually since 1963 and thereabout, and it was at its maximum in 1967 irrespective

of colicin type. Only strains of colicin type 14, which appeared for the first time in 1963 showed the maximum from the beginning. In the transition stage of resistance acquisition, it was possible to use resistance pattern as a subsidiary epidemiological marker in combination with colicin type provided that epidemics had been caused by colicin types 6, O, 8, etc. Two examples of application of this combination use were presented ; the first case deals with epidemiological analysis of a mass outbreak, and the second with epidemiological connection among many epidemics in a district within a definite period of time.

Introduction

The dysentery morbidity rate per 100,000 population in Japan for the past seventeen years, 1950–1966, had fluctuated between 50 and 130.[6] It decreased from 1967 on, and at last revealed a lowest peak, 17.5, in 1968. In connection with this state of affairs, the serogroup and the sero-type relation[6,7] of the organisms has brought about a marked change; for *Shigella sonnei* stood first in 1964 predominating over *S. flexneri* which had occupied first place up to that time. To be numerically concrete, of the total of 16,356 isolates of dysentery organisms from sporadic and epidemic cases in 1966, 14,487, 88.6 percent, were *S. sonnei*. Although clinically illness of this type of dysentery is mild and of short duration in general, it is a problem demanding special attention since mass outbreaks of this type are limited mostly to primary schools, kindergartens and day nurseries. In these circumstances, the study on colicin typing of *S. sonnei* and its practical application is fairly flourishing in Japan.

In this country, the colicin typing method of *S. sonnei* was devised early in 1959 by Naito et al. in this laboratory[29,30,31] independently of the method of Abbott and Shannon in England,[2] and by the former method, about 3,000 strains have been typed till now.[3,10,16,25] But, at present, the Naito's method is not of much help in epidemiology because of absolute predominance of type B strains typed by this method.[16,25] Although the Abbott and Shannon's method using a newly-arrived indicator set had been used since January 1966 in the authors' three laboratories, it was placed also in a position of considerable difficulty; for most of epidemic strains in Japan which corresponded mainly to Naito's type B were those of two groups where it was hard to separate either type 4 from type 14 or type 6 from type 11.[10]

Recently a timely work was carried out, tiding over the difficulty of the present time by Okada et al. of Saitama Prefectural Institute of Public Health.[33,34] They received some standard strains producing known colicins from Ozeki of the National Institute of Health, Tokyo, and analyzed "col" factor of each type of Abbott and Shannon's method, using a set of mutants resistant to known colicins produced by the standard strains. Most of colicin types of this method were those consisted of single or double

combinations of colicins E, I and K. When resistant mutants which were capable of detecting these colicins specifically are used additionally as indicators, it was possible to perform colicin typing by Abbott and Shannon's method more precisely and substantially, and fortunately to make a clear distinction between the colicin types in question.

After organization of a nation-wide working party consisting of no less than twenty bacteriologists (Aoki and Okada included), in June 1966 and discussions several times by the members, additional use of three or four strains among the mutants as indicators was publicly recommended in the name of the Ministry of Welfare in March 1967. In this paper, the authors will introduce this method using enlarged indicators, reporting the typing results of 3,686 strains of *S. sonnei* obtained by this method together with additional reports on biochemical types and drug-resistance patterns in a part of those strains.

This study on colicin type and biochemical type was done in the hope of making clear the distribution and the annual change of them in Japan, especially in West Japan, and comparing their results with those reported from the other part of Japan and from European countries. Only the results of resistance pattern were discussed, being in a different position, whether or not one can attack any significance as a supplementary epidemiological marker to it. As the factor responsible for drug-resistance, the resistance transfer factor, is believed to be a kind of episomes similarly as colicinogeny factor, it is natural to consider so. Indeed, Japan in the years 1962–1966 was just in a suitable state of things, during which a few strain manifesting sulfa-sensitivity or only sulfa-resistance had remained and multiple resistant strains to streptomycin, tetracycline and chloramphenicol had gone on increasing.

Material and Methods

Strains to be typed. Totaling 3,686 *S. sonnei* strains, consisiting of 947 strains isolated from sporadic cases and of 2,739 strains of 138 epidemic case origin, were used for colicin typing. Approximately 85 percent of them were isolated in prefectures Nagasaki, Fukuoka and Yamaguchi, and they were made the main subject of study on annual fluctuation of colicin type. The rest were collected from all quarters of western Japan, excepting Yamagata of the north-east districts. In these strains, 39 exotic strains have been included which consist of 20 strains sent from Taiwan, 13 strains from Hong Kong, five from Korea and one from Manila, the Philippines. Most of the strains isolated in a period from 1958 to 1965 were those stocked in cooked meat medium in Department of Bacteriology, Nagasaki University School of Medicine. The stability of colicin type in the course of preservation has been confirmed by Aoki et al.[10] using a restricted number of strains isolated in 1959. For determination of biochemical

type by Gillies's method[20] and of drug-resistance pattern, 328 and 1,875 strains were used respectively. Most of strains were selected from those used for colicin typing, but, especially in the case of drug-sensitivity tests, there were a fair number of strains collected from other prefectural institutes.

Indicator and type strains. Fifteen indicator strains for Abbott and Shannon's original method, 15 type strains for testing adequacy of the indicators within the limits of types from 1A to 13, were received from Dr. J. D. Abbott in December 1965. The resistant mutants used as additional indicators were: Row/E, Row/I and Row/K originated from *E. coli* K-12 Row, and K-12-30/I from *E. coli* K-12-30 (col E_1). These four strains, called K-12 mutant series, were presented by Okada together with type strains 4A, 9A, 13A and 14 (named Kurosu, Tokyo-to 75, Kurosawa, and Usukura respectively). Colicin types 4A, 9A, and 13A are new ones advocated by Okada et al.[34]; they assumed different patterns when K-12 mutant series were applied, while these strains were judged to be types 4, 9 and 13 respectively, provided the convenient set of Abbott and Shannon's indicators were used. Among the additional indicators, strain Row/K which is useful only for differentiation of type 1A and type 1B, can be omitted for routine purpose.

Colicin typing method and media. The method did not differ materially from that advocated in Dysentery Reference Laboratory in England, which was described briefly in Hart's paper[22] and, afterwards, notified in detail by her informally.[4] Only in following points the authors's method varies from the original : 1) rabbit or ox blood —— the latter was to be desired according to a comparative study of Fujise[17] —— was substituted as a component of medium for detecting colicin production, because horse blood is not easily available in Japan at present, 2) Dorset egg slope was not always used for pre-cultivation of test organisms, but was used for comparatively short(two or three months) preservation of strains before typing and for maintenance of indicator and type strains,[26] and 3) all media and chemicals used, those for fermentation and drug-resistance tests as well, were mainly Japanese products.

Nine routine indicators according to Hart[22] and three or four additional K-12 mutants were used simultaneously. An enlarged scheme for colicin typing (called Japanese modification hereafter) is shown in Table 1. In this table there is an obscure point as to the growth-inhibition pattern of K-12 mutants, because type strains "type 15", a new colicin type described by Gillies and Brown,[21] has not yet come into the authors' possession.

Although the greater part of the strains were typed by this Japanese modification, only 497 epidemic strains isolated in Fukuoka Prefecture were typed by Tokiwa, one of the authors, for their ability to produce standard colicins after Fredericq. As has been reported already by Tokiwa et al.,[41] colicins produced by type 1A, 2, 4, 6, 12, and 14 of Abbott and Shannon's method were identified with K, I, I, E_1, E_3, and

Table 1. An enlarged scheme for colicin typing of *S. sonnei* (Japanese modification)

Indicator strains	Colicin type																				
	1A	1B	2	3	3A	4	4A	5	6	7	8	9	9A	10	11	12	13	13A	14	15	O
56	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	−	−
17	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	−
56/56	+	+	−	+	+	−	−	+	+	−	−	+	+	+	−	+	+	+	+	−	−
2	+	+	−	+	+	+	+	+	−	−	+	+	+	+	−	+	−	−	+	+	−
R 6	−	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	−	−
M19	+	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−
2/7	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	+	+	−
R 5	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Row	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−
Row/E	+	+	+	+	+	+	+	+	−	−	−	+	−	+	+	−	−	+	+		−
Row/I	+	+	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+		−
Row/K	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+		−
K12·30/I	+	+	−	+	+	−	+	+	−	−	+	+	+	+	+	+	−	−	−		−

E_1+I, respectively. This is a measure for confirmation of colicin types in place of using K-12 mutant series.

Tests for biochemical type. On referring to a considerable amount of literature[20)32)35)36)37)38)39)40)], 328 or 300 *S. sonnei* strains, including indicator and type strains, were tested as to their fermentative activities on xylose, rhamnose, melibiose, raffinose, and salicin, as well as for beta-galactosidase reaction. The substrates, exclusive of Ortho-nitrophenyl - beta - D - galactopyranoside (ONPG), were prepared as one percent solution in peptone water containing 0.2 percent BTB and distributed in screw-capped tubes with an internal diameter of 13mm. After inoculation of an overnight culture with a nichrome-loop with an internal diameter of 2 mm, incubation was carried out at 37°C for 21 days, and the culture examined every 24 hours for evidence of acid production. The ONPG reaction was carried out by a modification of Kuwabara[24)], in which a small dose of the substrate is enough.

Tests for sensitivity to sulfa-drug and antibiotics. The plate serial dilution or/and the disk method were employed to test the sensitivity of 1,875 *S. sonnei* strains to six drugs : namely, sulfisomidine or sulfisoxazole(SA), streptomycin (SM), tetracycline(TC), chloramphenicol (CP), erythromycin(EM), and kanamycin (KM). Media used for the both methods were the Muller-Hinton medium(for SA) and heart infusion agar (for the others). The "Eiken" disks used here are made up each of three pieces of varied SA or antibiotic concentrations, and the amounts of the drugs contained in the disks of highest value are as follows : SA 300mcg, SM 50mcg, TC, CP and KM 30mcg, and EM 10mcg. In the disk method strains are recorded as "resistant" or "R", if they showed no growth-inhibition even in the mentioned highest concentrations. In the case of dilution

method "resistant" was recorded on the basis of the lowest concentrations of the drugs were : SA 3000mcg/dl, SM, TC and CP 100 mcg/ml, and EM and KM 50 mcg/ml.

Results

The results of colicin typing of 3,686 strains in all, arranged in order of the number, were: type 14, 1,770; type 6, 994; type O, 409; type 4A, 128; type 8, 120; type 13A, 95; type 4, 56; type 2, 26; type 1A, 15; types 9A and 13, 11; types 9 and 12, 9; type 3, 7; types 3A and 5, 6; type 1B, 5; type 11, 2, and 7 unclassifiable strains. It may safely be said, in a way, that types 14, 6, and O, accounting altogether for about 85 percent of all strains, are three main colicin types in Japan.

These results of 3,686 strains, however, are not pertinent to the investigation of geographical distribution and annual fluctuation of colicin types, because strains isolated from epidemic cases were exceedingly superior in number as compared with those from sporadic cases—— by 2,739 epidemic strains to 947 sporadic ones. Accordingly, the data concerning epidemic strains were so re-arranged that any one strain may represent all the isolates from an incident. That did well in case of an epidemic due to strains with the same colicin type, but, to speak the truth, there was a good number of instances where one or more colicin types other than a leading one were found in a mass outbreak case, especially when an exhaustive carrier detection was made.

In the latter case, one or more strains with different colicin types were selected in addition and included in the data of this study. Thus, 237 strains were checked from 138 incidents, and they were used as main materials for this study together with 947 strains originated from sporadic cases.

In Table 2 is given colicin type distribution of the mentioned 237 and 947 strains, 1,184 strains in total, by localities and years. Eighteen colicin types have been demonstrated in this table, among them types showing percentages less than eight, uc (unclassifiable) strains as well, have been collected in a colum, and they will be out of discussion in this report, excepting types 4A and 9 which are geographically in a peculiar position.

The mentioned three main colicin types have maintained their superior position and ranking even by this re-arrangement of the data ; type 14 occupies the first position (496 out of 1,184 strains, 40.9 %), type 6 ranks next (357 strain, 33.1 %), and type O (111 strains, 9.4%) follows them. The ranking under the type standing the fourth place was fairly disordered. It is due mainly to predominance of strains isolated from epidemic cases, for example of a case in extremity, 88 strains of type 4A have been obtained from four mass outbreak cases occurred in Fukuoka Prefecture in 1967, and type 4 and type 9 are similarly circumstanced.

Looking at these data from view-point of the interregional type distribution and

Table 2. Annual and interregional type incidence of colicin types

Region	Year	Colicin types										Total
		2	4	4A	6	8	9A	13A	14	O	Others	
Nagasaki	1958				5	3				3		11
	1959				23	26				21	1B : 3 12 : 1	74
	1962				34	8				1	uc : 1	44
	1963				40	27				3	uc : 1	71
	1964				26	1			1	4		32
	1965				2	1	1		4		1A : 1 3A : 1	10
	1966				16	4		5	257	5	1A : 1 12 : 1	289
	1967				20		1	2	88	3	13 : 1	115
	1968				19			1	3			23
Fukuoka	1960				19					3		22
	1961				21					6		27
	1965								2	1		3
	1966	3	4		6			2	18	3	1A : 2 9 : 1 11 : 2 uc : 1	42
	1967			4	7				11	1		23
	1968			6	4			2	11		1A : 1 13 : 1	25
The other prefectures of Kyushu	1960				1					1		2
	1961				9	4				3		16
	1962				18						uc : 1	19
	1965				2			3	27			32
	1966	6			20			2	27		3A : 2 5 : 3 12 : 4	64
	1967				8						1A : 1	9
Yamaguchi	1963		2		2					3		7
	1964		3		5				2	8		18
	1965	1			2				5	4	3 : 1 13 : 1	14
	1966	1	1		7				9	6	3 : 1 3A : 1 13 : 3 uc : 1	29
Other prefectures	1963				7				1	15	13 : 1	24
	1965				13					8	3A : 1 12 : 1	23
	1966	4	8		10	4		10	30	5	3 : 1 3A : 1 13 : 2	75
	1967				1							1
	1968							1				1
Korea					3					2		5
Taiwan					4	14					12 : 2	20
Hong Kong					3	1	8			1		13
Manila										1		1
Total		15	18	10	357	93	10	28	496	111	39	1184

of the annual changes in types, the following tendencies can be recognized : (1). Type 6 and type O were predominant up to 1964 in every district, and only four strains of type 14 were encountered in the corresponding period. (2). In 1965, however, 11 strains of type 14 appeared anew in prefectures of Nagasaki, Fukuoka and Yamaguchi, and this type increased abruptly in various quarters

from the next year onward. On the other hand, the number of types 6 and O reduced about to two thirds or by half from 1965 on (strains on type 6 are given as 212 before 1964 against as 135 since ; 71 against 36 in the case of type O). This trend concerning mutual relation of the three main colicin types seemed to be especially prominent in the results obtained in Nagasaki and Fukuoka Prefecture. (3). Type 8 could be detected at a considerable high rate in Nagasaki in 1959 and 1963. This type, types 2, 4, 13, and 13A as well, was considered to be comparatively rare but to characterize some localities in the western Japan in those days.

There is a great deal interest in detection of type 8 in Taiwan in 1965 and of type 9A in Hong Kong (isolation year is not clear). Strains of these two types form severally two thirds of the strains of *S. sonnei* presented by Taiwan Serum Vaccine Laboratory, Taipei and by Department of Microbiology, University of Hong Kong through the courtesy of Dr. S. T. Hsu, Director of the Laboratory and of Prof. C. T. Huang, and that they were considered all to be strains from sporadic cases, judging from isolation date and designations on lists. As for type 9A characteristic in Hong Kong, only two strains of it have been found in Nagasaki Prefecture in 1965 and 1967; one of them, detected in 1967, was isolated from a woman living in Sasebo City who had diarrhea on her return trip from Hong Kong by a plane. The number of strains isolated in Korea and Manila, the Philippines was so small that there is no scope for discussion regarding colicin type.

Fermentation studies were carried out for the purpose of investigating whether the mentioned colicin types and the biochemical types basing on fermentation reaction, for example those advocated by Gillies (1965)[20] and by Szturm-Rubinsten (1963, 1964),[38][39] are correlated each other, or not, and at the same time for investigation of the geographical distribution of the biochemical types. Accordingly, 19 type strains of each colicin type for the Japanese modification and 13 *S. sonnei* indicators of known colicin types for Abbott and Shannon's method were used as the most dependable materials for the first purpose, and 274 epidemic strains isolated in Japan and the neighboring countries, two Ewing's standard strains for *Shigella* classification (Nos. 33 and 34) in addition, mainly for the second purpose. The studies are now in progress, and latest findings, including data on 20 strains received from Dr. Ziesché in Wernigerode, East Germany and the results obtained by using salicin as a substrate in addition, are scheduled to be presented in a paper of Aoki, Iwanaga (formerly Yakushiji), and Ikeda (1966)[11]. The following summary is quoted from this report.

"Biochemical typing of *S. sonnei* by the scheme of Gillies and of Szturm-Rubinsten was performed using 328 and 300 strains with known colicin types respectively. One of the biochemical types established by Gillies which ferments raffinose and melibiose slowly, and the type "a" by Szturm-Rubinsten were prevalent in Japan and its neighboring countries. As both the types mentioned

above were almost equally distributed in colicin types 6, 8, 9A, 13A, 14, and O, it has been convinced that there is no relationship between biochemical types and colicin types as far as these materials concern. In colicin type 12, however, there were several types showing various fermentation patterns. It is noteworthy in this case that xylose-positive types prevailed over against xylose-negative ones in Europe, and vice versa in Asia. In addition, 51 strains were tested for salicin fermentation, among them three out of five strains isolated in Korea were proved to be fermentors of this glucoside. On the basis of the results obtained and in reviewing the literature, the authors had several discussions about the geographical distribution of biochemical types and about the present opinion as to taxonomic significance of salicin fermentation test."

Table 3 lists the results of 308 strains excepting 20 European strains, separating the epidemic strains from the standard strains. As shown in this table, 31 strains out of 32 type and indicator strains were determined as type RM (rhamose and melibiose positive but xylose negative) during the fixed 21-day observation period ; a pattern XRM was limited to the remaining one, a type strain of type 12, and in this case, acid production from xylose was noted in the next day after inoculation. Although there were no strains showing the biochemical type XRM in the other epidemic strains of this colicin type, it can be said, at any rate, that type 12 is a peculiar colicin type which manifests highly variable fermentation reactions. Fermentation pattern of the other colicin type strains was determined for the most part as RM, whereas colicin types 6, 8, 14, and O, abound comparatively with samples, often trended to develope other fermentation patterns, especially M. In short, only type 12 seems to be discussed

Table 3. Fermentation reactions and typing of *S. sonnei* according to Gillies, classified by colicin types

X	R	M	Colicin types																			Total
			1A	1B	2	3	3A	4	4A	5	6	7	8	9	9A	10	11	12	13	13A	14	0	
+	−	−																2					2
+	+	−																			1		1
+	−	+	1																			1	2
+	+	+																1^{1}					1^{1}
−	+	−									1		3					3				1	8
−	+	+	3^{1}	5^{2}	8^{7}	2^{2}	5^{1}	1^{1}	1^{1}	1^{1}	57^{2}	1^{1}	55^{5}	1^{1}	11^{1}	1^{1}	1^{1}	3	1^{1}	12^{1}	20^{1}	51	240^{31}
−	−	+	1								17		6					1		1		5	31
−	−	−									3		1					16				3	23
Total			5^{1}	5^{2}	8^{7}	2^{2}	5^{1}	1^{1}	1^{1}	1^{1}	78^{2}	1^{1}	65^{5}	1^{1}	11^{1}	1^{1}	1^{1}	26^{1}	1^{1}	13^{1}	21^{1}	61	308^{32}

X=xylose, R=raffinose, and M=melibiose.
Index numerals indicate those of type and indicator strains for Abbott and Shannon's colicin typing method, forming a part of the fugures listed.

separately from the other colicin types from a view-point of sugar fermentation.

As for Szturm-Rubinsten's biochemical types, which can be determined by combinations of ONPG, xylose and rhamnose reactions during 14 day observation, 280 strains of known colicin types were placed at the examination. They were all determined as type "a", a type characterized by positive ONPG and rhamnose and negative xylose, excepting following 12 strains : each five strains of types "e" and "f" and two strains of type "g". It goes without saying in this case also, that biochemical type of *S. sonnei* bears no relation to colicin type. But, as for strains belonging to colicin type 12, inquiry must be reserved similarly, because five out of the mentioned 12 exceptional strains have been enrolled to this colicin type.

The drug-resistance tests with sulfa-drug, streptomycin, tetracycline, chloramphenicol, erythromycin, and kanamycin were performed on 1,875 out of 3,647 epidemic strains isolated in Japan and on 39 strains received from Korea, Taiwan, Hong Kong, and the Philippines. The results obtained were expressed simply by arranging designations R (resistant) and S (sensitive) in order of the drugs as mentioned above. Of designation R in connection with drug concentrations the authors have noted already. "S" has been used here not literally, but in the sense of "not R".

Leaving time and place of isolation of the strains out of consideration, general view of drug-resistance pattern classified into four groups according to colicin type

Table 4. Durg-resistance pattern divided into classes of colicin type

		Type 14	Type 6	Type O	Others	Total	Exotic strains
	R R R R	955 (98.0)	257 (46.6)	56 (27.2)	55 (38.1)	1323 (70.6)	19
	R R S R	10	16 (2.9)	3 (1.5)	2 (1.5)	31 (1.7)	2
Group 1	R S R R		8 (1.4)	1	16 (11.1)	25 (1.3)	2
	R R R S	1	4			5	
	S R R R		1			1	
	R S R S		2	1	2 (1.5)	5	
	R R S S			4 (1.9)		4	7
Group 2	S R R S		1			1	
	S S R R	1				1	
	R S S S	4	191 (34.8)	114 (55.4)	56 (38.8)	365 (19.5)	6
Group 3	S S R S		8 (1.4)			8	
	S R S S			2		2	
	S S S S	2	64 (11.6)	25 (12.1)	13 (9.0)	104 (5.5)	3
Total		973(100.0)	552(100.0)	206(100.0)	144(100.0)	1875(100.0)	39

R (resistant) and S(sensitive), in the order of sulfa-drug, streptomycin, tetracycline, and chloramphenicol. Figures in parentheses are percentages (those less than 1.0 omitted).

of 1,875 Japanese strains from the neighboring countries is shown first in Table 4. In this table the presentation of results on erythromycin and kanamycin has been abridged for want of R in all test-strains.

The strains could be classified further into 14 resistance patterns consisting of various combinations of R and S. They are arranged in order of decreasing number as follows: RRRR, RSSS, SSSS, RRSR, RSRR, etc. Drug resistance patterns other than these five noted here were so small in number that they are little worth consideration. Although RRRR stood first on the whole, it ranked next to RSSS so far as colicin type O and "other types" are concerned. At any rate, it is noteworthy that types 14 and O represented two extremes of the resistance pattern distribution. The results on 39 exotic strains have been noted as an appendix to the table for reference.

Annual changes in drug-resistance of all examined strains as a whole and those divided into colicin types during a period from 1959 to 1967 are shown diagramatically with distribution percentages of resistance strains in Figure 1. The resistant strains in this figure mean those belonging to the group 1 in Table 4 which consist mainly of the four-R strains and partly of the three-R strains. Although there were no resistant strains of this meaning in the isolates in 1959 and 1960, there could be found three RRRR and six RSRR in 30 strains of type O in 1961, and one each RRRR and RRSR in type 6 and in unclassifiable strains in 1962. In 1963, the percentages of type 6 and "other types" were raised markedly, and year by year since. Type 14 which appeared first in 1963 showed all RRRR pattern from the first and

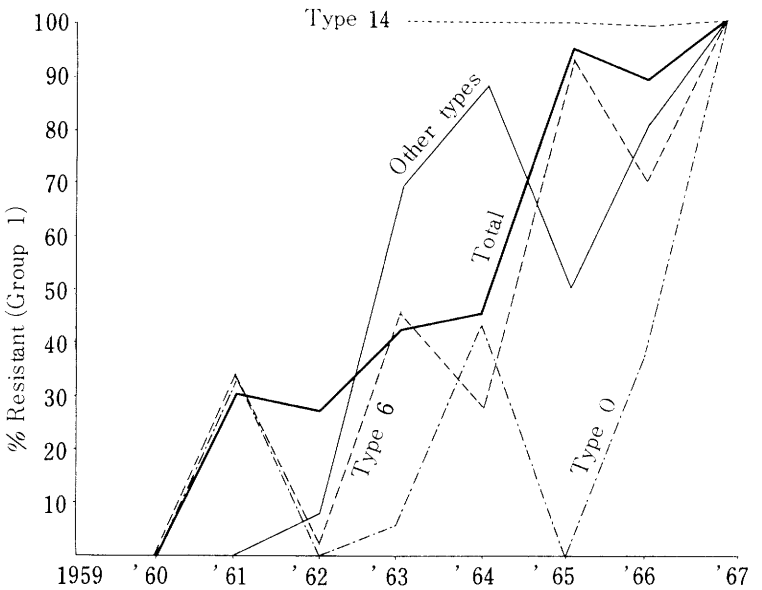

Fig. 1. Changes in drug-resistance of *S. sonnei*, 1959–1967; the yearly fluctuation of group 1, in total and separately by colicin types

maintained this situation till 1967. On the contrary, the resistant strains of type O could be not detected in the years 1960, 1962 and 1965, but amounted up to the highest limit in 1967.

As it is said that an acquisition of drug-resistance in gram-negative enteric bacteria especially the one related to sulfa-drug, streptomycin, tetracycline and chloramphenicol is attributable to a resistance transfer factor RTF, a kind of episomes like temperate phage, F factor and colicinogenic factor (Watanabe, 1963), it is conceivably possible to use drug-resistance as an epidemiological tool independently of colicin type in *S. sonnei*. In this respect, the western part of Japan in the years 1962–1966, especially 1963 and 1964, in which drug-resistance of *S. sonnei* happened to be in a turning-point, seemed to be a suitable field for investigation of this possibility.

As mentioned already, there were 138 incidents of dysentery epidemic in the entire scope of this report. Forty-four cases among them, however, were regarded as incidents occurred in 1967. As in this year both the groups of strains isolated from mass outbreak cases and those from sporadic cases showed almost all the resistance pattern RRRR, there was no room for discussion about epidemiological significance of the sensitivity tests. Thirty-five incidents among the remainders, were not available also in epidemiology because of use of the least

Table 5. Sonne dysentery incidents in Yamaguchi Prefecture (July 1963–February 1965)

No. of incid.	Time	Institution and locality	No. of patients	No. of strains	Colicin type (resistance pattern)
18	July, 1963	Tachibana, Oshima	62	3	O (R S S S) : 3
19	Oct., 63	a hotel, Hofu	9	8	6 (R S S S) : 8
20	Dec., 63	a factory, Shimonoseki	66	43	6 (R R R R) : 40 O (R S S S) : 2 14 (R R R R) : 1
22	〃	a day nurs., Yanai	115	12	O (R S S S) : 12
23	Jan., 64	an inf. sch., Kudamatsu	10	8	14 (R R R R) : 6 6 (R R R R) : 1 6 (R R S R) : 1
24	〃	Kaminoseki, Kumage	120	10	O (S S S S) : 10
25	〃	a prim. sch., Yamaguchi	12	12	O (R S S S) : 10 O (R R S S) : 1 O (R R R R) : 1
26	〃	an inf. sch., Yamaguchi	12	12	O (R S S S) : 11 O (S S S S) : 1
28	May, 64	a day nurs., Onoda	18	11	O (R S S S) : 11
29	July, 64	a prim. sch., Toyoura	23	11	6 (R R R R) : 9 6 (S S S S) : 1 O (S R R R) : 1
30	Sept., 64	Yashiro, Kumage	26	4	6 (R R R R) : 2 14 (R R R R) : 2
31	Dec., 64	a day nurs. A, Mine	65	13	O (R R R R) : 13
32	〃	a day nurs. B, Mine	28	22	O (R R R R) : 22
33	Feb., 65	a day nurs., Shmonoseki	25	17	14 (R R R R) : 15 O (R S S S) : 2

number of isolated strains, lack of drug-sensitivity tests, or by other reasons. After all, following 48 incidents remained to be somehow available for epidemiological analysis, which were classified into four groups in accordance with colicin types controlling respective epidemic cases. (1) Seventeen incidents caused by type 14 alone, or by a combination of it with any other type of the minority, and each one incident caused by types 2, 4, and 5, respectively. As the isolates from these 20 cases showed the resistance pattern RRRR in all or in an absolute majority, the determination of resistance pattern is no more than a certification of the colicin types. (2) Ten incidents due to type O consisting of eight cases marked with RSSS and two cases with RRRR. (3) Sixteen incidents of type 6 which consisted of eight RRRR, six RSSS, and two SSSS. (4) Two incidents of type 8 ; one RSSS, the other being mixed with RSSS and SSSS. Of the incidents of the groups from (2) to (4), it has been proved that epidemic cases due to the same colicin types can be divided into two or three according to resistance patterns.

Details on every incident occurred in Kyushu in 1959–1964 and in Chugoku-Shikoku in 1963 and 1964 have been reported already in the papers of Jinnouchi and of Morino respectively, and the remainder will be reported by Miyahara on some future occasion. Two examples which brought about the disired epidemiological effect by combination of colicin type with drug resistance pattern are noted as follows :

The first case bears on epidemiological analysis of an incident which occurred in a day nursery in Nagasaki City in the last five days of January, 1962. Fourteen out of 79 admitted children were attacked with the disease, and this epidemic was presumed, by information obtained by inquiry, to be generalized infection due to feeding. Fourteen strains isolated from the patients and other children without symptoms were determined to be made up of nine strains of colicin type 8 with the resistance pattern RSSS, two strains of the same type with SSSS, and one each type 6 with RSSS, type O with SSSS, and "unclassifiable" with RSSS. By examination of the families, five carriers of type 8 (RSSS) were detected only from family members of four children carrying the mentioned type 8 strain with RSSS. One of day-nurses, being a carrier of type 6 strains with the resistance pattern RSSS, could be asided from the epidemic. By examination of employers of four food deliveries, seven carriers were found. Strains obtained were : type 6 (RSSS) from the deliveries A, B and C, and one strain of type 8 (RSSS) from the delivery D which supplied fried fish pasts on that day in question. Evidently this epidemic was caused mainly by *S. sonnei* of colicin type 8 with the resistance pattern RSSS through the medium of contaminated fried fish pasts.

The second example concerns with a great-sphere epidemiology, in other words with the geographical distribution and the annual change of colicin type in a district. As mentioned earlier, drug-resistance pattern of strains belonging to type 6 and type O has changed from

SSSS to RRRR with the lapse of time, while that of type 14 was RRRR from the first, and that in cases of the former two colicin types, the change SSSS→RSSS was slow in moving but RSSS → RRRR suddenly. This state of affairs can be seen in Table 5 in which the results on 14 mass outbreak cases of dysentery caused by colicin types 6, O and 14 in Yamaguchi Prefecture during a period from July 1963 to February 1965 are summarized. They were classified into six groups according to the governing colicin type as follows: one incident caused by type O (SSSS), five type O (RSSS), two type O (RRRR), one type 6 (RSSS), three type 6 (RRRR), and two type 14 (RRRR). These incidents have been arranged in the expected order with the lapse of time, but there was an exceptional case, No. 24, which occurred in a detached island in the Inland Sea and was characterized by type O with the resistance pattern SSSS.

Discussion

It is worth specially mentioning in this study that there is no longer need to use the ambiguous type designations 6/11 and 4/14 described in the previous report of Aoki et al.[10] The difficulty in distinction of type 6 and type 11 has been pointed out already by Abbott and Graham[1] and by Brandis and Meyer-Nieberg.[13] Aoki, one of the authors, also has heard that Dysentery Reference Laboratory in England is perplexed with this problem (Hart, personal communication ; Aoki[4]). The analogous difficulty concerning types 4 and 14 came to the knowledge of Japanese workers including the present authors in 1967. Moreover, there is a report of Mulczyk et al.[28] who could type only a part of *S. sonnei* strains isolated in Poland by the method of Abbott and Graham[1] and Gillies.[18] The so-called Japanese modification of Abbott and Shannon's method using three or four resistant mutants induced from *E. coli* Row and K-12-30 as additional indicators, brought these problems to a satisfactory solution. It is a finding of the present study that epidemic strain of type 11 was hardly found in Japan, though the type strain 11 was exactly distinguishable from type 6. That "unclassifiable" strain by this method was very rarely to be met with, is also deserves emphasis.

The results obtained by this method on all 1,184 *S. sonnei* strains representing "foci" including 39 strains isolated in Korea, Taiwan, Hong Kong, and Manila were : 496 strains of type 14 (40.9%), 357 strains of type 6 (30.1%), 111 strains of type O (9.4%), 93 strains of type 8 (7.9%), 28 strains of type 13A (2.4%), etc. There is a marked difference in type distribution between the results of this study and those of British investigators (Abbott and Graham,[1] Barrow and Ellis,[12] Cook and Daines,[14] Hart,[22] Gillies,[18][19] Farrant and Tomlinson[15]) who showed a predominance of types 7 and O and a low incidence of types 1A,2,4,8,13, etc. Among them, Abbott and Graham and Hart have used some foreign strains collected from some

different countries in 1960–1962. Their information, in which types 6 and O were comparatively more numerous, seems to support to a certain extent the data presented by the present authors in approximately the same period. Type 14 had been noted by Gillies since 1959, but it was described by him as a new type in 1964 together with his typing results from 1959 to 1961.[18] At that time, only 12 strains of this type were found in England among 1,056 strains tested.

There are two forthcoming reports on colicin type distribution in the European Continent: the papers of Mulczyk et al. in Poland[28] and of Brandis and Meyer-Nieberg in Germany[13], who have noted comparatively large number of "untypable strains" (strains which do not produce inhibition of any indicators, namely type O designated by Hart and used by Japanese workers) and, in the scope of typable strains, an occurrence of types 11, 6 and 2 in the former case and types 2, 6, 8, and 11 in the latter case. After all, it can be said that a decided difference in colicin type existing between Japan and European countries is attributable to nonexistence or minority of type 14 there which abounds here.

In Japan, after organization of a nation-wide working group on colicin typing of *S. sonnei*, many reports have been made of the existing colicin types in various districts. According to Aoki,[8] one of the authors and a chief investigator of this working group, who compiled recently a report on colicin types of 8,321 strains (foci) isolated in all Japan during a period from 1953 to 1968, the distribution of colicin types is common to the whole country so far as the three main types, types 14, 6 and O, are concerned. Type 14 among them which are rare to find in Europe have been detected by 34.6 percent in this report of Aoki and by 40.6 percent in the present report of Aoki and others on strains isolated mainly in the western part of Japan. In this authors' opinion, the difference in colicin type seems to be an intercontinental or international problem, not interregional in a country, especially in an island country like Japan. In this respect, the authors place a high value on the typing results of strains isolated in Taiwan and Hong Kong, small in mumber as they are.

Apart from that, there was some difference in the minor colicin types such as type 8 and type 13A within the boundaries of Japan ; type 8 was comparatively numerous in Kyushu especially in Nagasaki Prefecture, and type 13A in Shikoku. The local color like this and annual changes of each colicin type from a nation-wide standpoint have been described in the report of Aoki.[8]

The usefulness of colicin typing as an epidemiological measure in the case of dysentery outbreak has been described already in some papers of this Department. In the present report, a possibility of applying drug-resistance pattern as a subsidiary epidemiological marker in combination with colicin type was discussed. This possibility is, however, conditional on colicin type, time of enforcement, general state of epidemiological affairs, etc., and in this report, only the records on the annual changes of resistance pattern classified by colicin

type, colicin type and resistance pattern of epidemics occurred in Yamaguchi Prefecture during a period from July 1963 to February 1965, and an example of epidemiological analysis on an incident occurred in a day nursery in Nagasaki City, July 1962, have been noted and explained.

Studies on fermentation pattern of *S. sonnei* according to Gillies[20] and Szturm-Rubinsten[38][39] resulted in the predominance of a type, the xylose negative but raffinose as well as melibiose positive strain by Gillies' method, and of type "a" by Szturm-Rubinsten's method. Both the biochemical types bear no relation to colicin type each, but it deserves special mention that atypical strains showing various fermentation patterns appeared most frequently among strains of colicin type 12. The application of biochemical typing as well as determination of resistance pattern to minute epidemiological analysis of a group of regional mass outbreak cases or of any incident of itself, is conceivably possible as far as it is performed together with colicin typing.

After the completion of this manuscript, it was informed that results of colicin typing of *S. sonnei* isolated in Taiwan in the years 1965–1968 were reported by Liu et al. in Taiwan Serum Vaccine Laboratory and Fu-nin University to the 16th regional meeting of the Formosan Medical Association at Kaohsing. Their results were informed personally by Dr. Shu-Tao Hsu, Director of the Laboratory, while this paper was in the press. Using 33 strains mentioned above, the authors typed them as follows : 27 strains of colicin type 8, five of type 6, and one of type 12. The predominance of type 8 in Taiwan lends support the present authors' (Aoki and others) finding.

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日本とその近隣諸国で分離された Shigella sonnei のコリシン型，生化学型および薬剤耐性パターン

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摘　　要

日本で改良され，標準法として一般に実施されている Abbott-Shannon の Shigella sonnei コリシン型別法（いわゆる型別部会法）を紹介した．本法は大腸菌K-12変異菌から誘導した抵抗変異株３ないし４株を原法指示菌に追加したもので，原法では区別が困難であった６型と11型，および４型と14型を明瞭に分け，また新コリシン型３種，4A，9A，13A を追加したものである．主として西日本で分離された，ホーカスを代表する1,148株の S. sonnei が本法による型別に供された．現在の日本では14型が首位，６型これに次ぎ，O型，８型，13A，４型，２型などがこの順に検出され，うち８型と13A は国内的に地理的分布上のかなりの差異を示した．ほかに近隣諸国分離の39株を型別したが，その成績は日本のそれと大いに差異があった．生化学的型別の結果は，78％までが Gillies の RM 型（キシローゼ陰性，ラフィノーゼ，メリビオーゼ陽性），96％までが Szturm-Rubinsten の a 型（ONPG陽性，ラムノーゼ，キシローゼ陰性）であった．この両型はともに12型以外の各コリシン型に平均的に分布し，12型においてのみ各種の糖分解型式を示すものが見出された．これらの菌株のサルファ剤と３種の抗生物質に対する抵抗性は 1963年頃から次第に高まり，1967年に至ってコリシン型に関係なくすべてが最高の抵抗を示した．ただし14型だけはそれが最初に現われた1963年には，４剤に対して既に最高の抵抗を示していた．この耐性獲得の途上にある時期では，６型，O型，８型などによる流行の場合に限つて，薬剤耐性パターンをコリシン型と組合せて一つの補助的な疫学的マーカーとして利用することが可能であった．この組合せ使用の二つの応用例を記述したが，一つはある集団発生の疫学的分析に関するものであり，もう一つは，ある地方ある期間内における各流行例間の関連に関するものであった．