VI-PHAGE TYPES IN SINGAPORE

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SYNOPSIS

Vi-phage types of Salmonella typhi have been studied. Types B1, D1 and A are found predominant in Singapore. Results of the phage-typing show that there is a variety of types and each of the outbreaks in the past two years was caused by strains of the same phage-type. The local strains are sensitive to most of the antibiotics tested.

INTRODUCTION

In 1926, Kristensen and Henriksen subdivided the typhoid bacilli into three biochemical types using the fermentation reactions of arabinose and xylose as a criterion. However, this technique proved inadequate as a tool for epidemiological studies. Later in 1938, Craigie and Yen introduced their phage-typing scheme of *s. typhi.* Since then, Vi-phage typing has been recommended for typing the typhoid strains as it provides essential information for epidemiological investigation.

This report is complied with data obtained from typhoid cultures collected in the past two years (October, 1975 to September, 1977) to show the common phage-types in Singapore.

MATERIAL AND METHODS

In Singapore as in other countries, the standard laboratory tests carried out for the diagnosis of typhoid are blood and stool cultures, Widal test and blood clot culture. In the case of a suspected carrier, urine culture is also included in the investigations.

Blood is cultured in trypticase soy broth added with 0.05% "liquoid" (sodium polyanethol sulphonate) and 0.5% thioglycollate, and examined after 24, 48 hours, and a week's incubation at 37 C. Stool is inoculated into Selenite broth and MacConkey agar plate followed by 37 C incubation overnight. Blood clot recovered from

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the blood used for Widal test is cultured in trypticase soy broth containing 1% ox-gall, preferably added with 1% streptokinase or trypsin. The culture is examined after 24 and 48 hours' incubation at 37 C. For Widal test, the tube agglutination method is used. Urine is cultured in Selenite broth and incubated at 37 C.

The Vi-phage typing technique is basically similar to that of Craigie and Felix (1947), which was recommended by Anderson and Williams in 1956. However, instead of spotting, the pre-dried agar plates are flooded with 2.5 hour-old broth culture of the test strain (approximate cell density 1.5 × 10^8 per ml.). The phages are then spotted on the lawn with a platinum wire loop of internal diameter 3 mm. After overnight incubation at 38.5 C, the Vi-phage type of the strain is determined by the characteristic lytic pattern produced by the phages on the inoculated plates.

RESULTS AND DISCUSSION

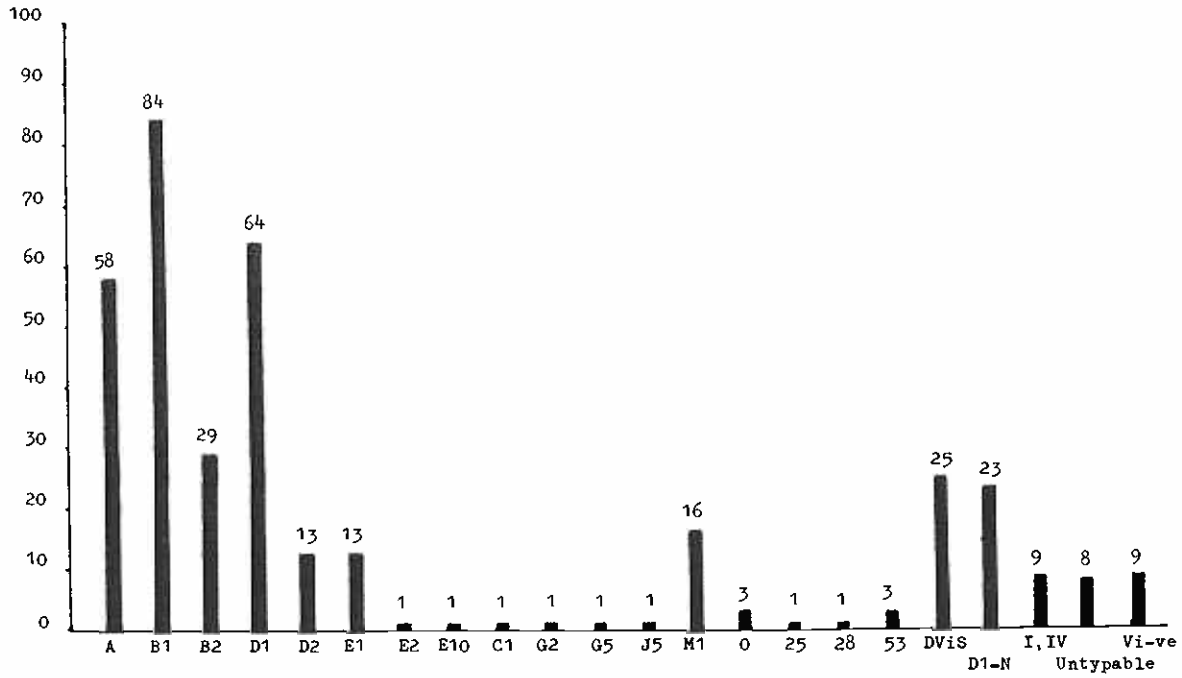
In an earlier report by Lam et al. (1973), it was shown that typhoid was endemic in Singapore with an average number of less than two hundred per year. However, there were occasional outbreaks throughout the year, but these were promptly dealt with by the health authorities.

Since the Vi-phage typing work started in February, 1977, the common phage types in Singapore between October, 1975 and September, 1977 (two years) have been studied (Table I and Graph I) and recorded in order of frequency as follows: B1 (23%), D1 (18%), A (16%), B2 (8%), DViS (7%), D1-N (6%), M1/D2/E1 (4%), I, IV/ untypable/Vi -ve (2%) and the rest are less than 1%. The figure within brackets for each phage-type indicates the percentage out of a total of 365 strains typed.

TABLE I. Distribution of Vi-Phage types of *S. Typhi* during a two-year period

	A	B1	B2	D1	D2	E1	E2	E10	G1	G2	G5	J5	M1	O	25	28	53	DViS	D1-N	I, IV	Untypable	Vi negative	TOTAL NO. OF CASES
(1975)																							
October	3	25	5	6														2		2	2	2	47
November	5	18	13	1	2													7				3	49
December	2	4	3			1	1								1	1		1	4		2	2	22
(1976)																							
January	3	8									1						1	1		3			17
February	4								1														5
March	3	1	1		1																		6
April	6	1		1	2	1												1					12
May		1											3								1		5
June	4											1						1			1		7
July	4	1						1									1						7
August	2			1		1																	4
September	3			6	1													2		1			13
October	1			2	1	1				1								2					8
November	2	1											2				1	3		1			10
December	4			2										1									7
(1977)																							
January	6	2	1	2	3	1							6					2					23
February		3	1	1		1							1										7
March	2	3	1	10	1								1					1	5			1	25
April	2	4	1	9	1	1												2	10	1			31
May		2		9									1						2			1	15
June		5	1	5	1								1						1	1	1		16
July	1		1	1		2								2					1				8
August		5	1			4															1		11
September	1			8									1										10
TOTAL	58	84	29	64	13	13	1	1	1	1	1	1	16	3	1	1	3	25	23	9	8	9	365

Graph I. Distribution of VI-Phage types of *S. Typhi* during a two-year period



Between September and December, 1975, an unusually high incidence of 118 typhoid cases were reported. The majority of the strains isolated belonged to type B1, but epidemiological investigations failed to trace the source of the infection as there was no common link among them. However, carriers have been detected (as shown in Table II) and this is essential in checking the spread of the infection. In 1976, seven members out of a Malay family of eight were infected with type A. In 1977 (January to September), three outbreaks were reported. In the first incident, five students from two secondary schools fell ill with typhoid. Epidemiological studies showed that these students used the same bus-stop and patronised the few food-stalls nearby. However, bacteriological examination of the food samples taken from these stalls did not recover the *S. typhi* which was type D1-N. The second incident occurred in Kolam Ayer area in which seven persons were infected with type B1. Between February and September of the same year, forty five persons and seven carriers were detected. The strains belonged to type D1. The cause was unknown.

According to the Singapore mid-year estimated population report for 1976, the total was 2,278,200 and the number of each ethnic group was as follows:

Malays	342,900	(15%)
Chinese	1,734,600	(76%)
Indians	156,500	(7%)
Others	44,200	(2%)

Since Singapore is a multi-racial country, the susceptibility to certain phage-types by each race was also studied. As shown in Table III, the following phage

TABLE II. Typhoid incidence in Singapore

Year	Total no. of cases	Outbreak	Total no. of carriers
1972	167		
1973	154		
1974	294	Jurong	13 females
			3 males
1975	500	Sporadic cases in five areas	2 females
1976	129	Bukit Kaki	1 female
			3 males
1977	204	Yio Chu Kang	3 females
			10 males

TABLE III. Distribution of Phage-types among ethnic groups

	(Malays)	(Chinese)	(Indians)	(Others)
A	22	28	7	1
B1	19	59	3	3
B2	5	24		
D1	9	51	4	
D2	2	9	1	1
E1	2	6	2	3
E2		1		
E10		1		
G1		1		
G2		1		
G5		1		
J5	1			
M1		15		1
O		3		
25	1			
28			1	
53	2	1		
DViS	7	15	1	2
D1-N	5	16	2	
I, IV	2	6		1
Untypable	1	7		
Vi -ve		8	1	
Total no. per group	78	253	22	12
Percentage of 365 cases =	21.4%	69.3%	6%	3.3%

pattern is obtained for each ethnic group:

Malays — A, B1, D1, B2/D1-N/DVis, D2/E1/53/I, IV.

Chinese — B1, D1, A, B2, D1-N, M1/DViS, D2, Vi -ve, Untypable, E1/1, IV, O.

Indians — A, D1, B1, E1/D1-N.

Others — B1/E1, DViS.

(For convenience, those phage-types which occurred only once during the two-year period have been omitted). The above results show that B1, D1 and A are the common types found in the three main ethnic groups. Racial susceptibility to certain phage-types can thus be ruled out.

As shown in Table IV, typhoid was commonest in the age-range from 5 to 54, with the largest number of cases found in the (15-24) group. This age-range represents the schooling age and working class of the population who have more chances of exposure to typhoid than the younger and older groups.

In the 1976 mid-year estimated population report, there were 1,162,000 males (51%) and 1,116,200 females (49%) in Singapore. During the two-year period under study, 223 males (69%) and 142 females (31%) were infected with typhoid (Table IV). Although Huckstep (1962) found that sex susceptibility to the infection was the same, the results obtained in Singapore seem to show an appreciably higher incidence in the males than the females, especially to phage-types A, B2, D1, D2, DViS and I, IV.

With the collaboration of the National Institute of Health in Tokyo (through the arrangement of "South-East Asia Medical Information Centre"), antibiotic sensitivity patterns of about three hundred *s. typhi* strains from Singapore were studied. Results showed that the local strains were sensitive to Chloramphenicol, Tetracycline, Streptomycin, Kanamycin and Amino-benzylpenicillin. The only recorded strain which was resistant to Chloramphenicol, Tetracycline, Streptomycin and Rifampin, was isolated from an imported case of typhoid from Saigon in 1974. However, this strain was sensitive to Ampicillin, Carbenicillin, Cephalothin, Co-trimoxazole, Gentamicin, Kanamycin and Polymyxin B.

From the Vi-phage typing of *S. typhi*, we have learned the phage-types common in Singapore. This is valuable in the epidemiological investigation of typhoid.

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TABLE IV. Age and sex distribution of Vi-phage types

(AGE)	A	B1	B2	D1	D2	E1	E2	E10	G1	G2	G5	J5	M1	O	25	28	53	DVIS	D1-N	I, IV	Untypable	Vi -ve	Total
Under 1	1	1	1																				2
1 — 4	2	2	2	2		1								1				2					12
5 — 14	11	18	8	4	5	6	1							1	1	1	1	5	6	2	1	1	72
15 — 24	21	31	9	20	1	2				1		1	11	1			2	6	14	3	4	3	130
25 — 34	14	16	6	17	4	2		1					4					8	1	2	1	2	78
35 — 44	4	7	3	4					1									2	2			1	24
45 — 54	3	5		11	2	2												2		1	2	1	29
55 — 64		2		4	1															1		1	9
65 — 74	2	2		2							1												7
76 — 84													1										
(SEX)																							
Male	33	40	20	56	10	7		1	1				7	1		1	2	18	10	8	4	4	223
Female	25	44	9	8	3	6	1			1	1	1	9	2	1		1	7	13	1	4	5	142

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REFERENCES

1. Anderson E.S. and Williams R.E.O.: "Bacteriophage Typing of Enteric Pathogens and Staphylococci and its use in Epidemiology." J. clin. Path., 9: 94-127, 1956.
2. Craigie J. and Felix A.; "Typing of typhoid bacilli with Vi-bacteriophage." Lancet, i, 823-827, 1947.
3. Craigie J. and Yen C.H.: "The demonstration of types of B. typhosus by means of preparations of Type II Vi-phage: Principles and techniques." Canad. Publ. Hlth. J., 29: 448-463, 1938.
4. Huckstep R.L.: "Typhoid Fever and other Salmonella Infections." pp. 49, E. & S. Livingstone Ltd., Edinburgh and London, 1962.
2. Kristensen M. and Henriksen H.C.D.: "Reactions Fermentatives Du Bacille Typhique et Leur Role Epidemioloque." Acta path. microbiol. scand. 3: 551-583, 1926.
6. Lam S., Monteiro E. and Sng E.H.: "A Review of *s. typhi* Infections in Singapore: 1969-1971." Asian J. Med., 9: 129-132, 1973.