

Phage types of *Salmonella typhi* isolated in Malaysia over the 10-year period 1970-1979

By M. JEGATHESAN

Division of Bacteriology, Institute for Medical Research, Kuala Lumpur, Malaysia

(Received 17 June 1982; accepted 21 July 1982)

SUMMARY

The pattern of phage types of 2553 strains of *Salmonella typhi* isolated over the 10-year period 1970-9 was studied. During the period 29 different phage types were encountered, not including the categories of 'untypable strains', 'degraded Vi-strains' and Vi negative strains. For the period as a whole, the commonest phage types encountered were A (20.9%), E₁ (14.8%), D₁ (10.3%), degraded Vi positive strains (10.3%), untypable Vi strains (7.3%), C₁ (7.1%), D₂ (4.4%), E₂ (3.9%) and type 25 (2.6%). There were phage types which appeared in the early years of the period and then disappeared (types B₂, D₉ and D_{1-N}). Others only made their appearance in recent years (K₁ and 53). Notable differences were also seen in the predilection of some phage types for certain geographical areas.

INTRODUCTION

Typhoid fever continues to be an important public health problem in Peninsular Malaysia as in many other developing countries. Typhoid is a notifiable disease in Malaysia and for the years 1964-76 the average number of reported cases per year was about a thousand. Except for one or two years, when higher than average number of cases had occurred, the endemicity of typhoid remained quite constant despite control activities. The annual incidence of reported cases varied from 8.5 to 12.7 per 100000 population (Lo, 1977).

Since its introduction by Craigie and Yen (1938), the Vi-phage typing scheme has become the method of choice for the epidemiological finger-printing of *Salmonella typhi* strains.

The detection of phage types of *S. typhi* prevalent in a country and indeed even in areas within a country is more than an academic exercise. The typing results obtained have in many instances facilitated the tracing of the route of transmission and detection of the infectious source (Ohashi, 1977).

Geographical distribution of phage types of *S. typhi* presents a very important background for the surveillance of typhoid fever. Its pattern is more or less specific for each country or geographical unit (Fukumi, 1977).

The Bacteriology Division of the Institute for Medical Research, Kuala Lumpur, Malaysia, started performing Vi-phage typing of *S. typhi* strains in 1971 with the setting up of the '*S. typhi* Vi-phage Typing National Centre'. This was done with the co-operation of the World Health Organisation and Dr. E. S. Anderson (Director, International Reference Laboratory for Enteric Phage Typing, Colin-

dale), who not only trained one of our senior laboratory technologists in the technique but also provided the full complement of the Vi-typing phages, type strains and methodology, (Singh, 1971).

This paper discusses the results of phage typing conducted on strains isolated throughout Malaysia during the 10-year period 1970-9.

MATERIALS AND METHODS

Strains phage-typed included those isolated in the Bacteriology Division of the Institute for Medical Research and others submitted by the pathologists from the hospital laboratories in the various states of Malaysia. Strains were also received from the Microbiology Departments of the University of Malaya (since 1974) and the Universiti Kebangsaan (since 1978).

The method of phage typing was as recommended by the International Reference Laboratory for Enteric Phage Typing, Colindale, England.

RESULTS

Table 1 shows the frequency-distribution of the different phage types by cases. Table 2 shows this distribution by foci. For the purposes of gauging the relative importance of each phage type, distribution by foci rather than by cases (gross type distribution) is considered to be a more reliable indicator (Anderson & Williams, 1956).

During the 10-year period 2553 isolates were typed. These came from 1558 foci. It is estimated that during this 10-year period about 10000 cases of typhoid were notified. Many of these would have been notified on the basis of clinical features and/or Widal reactions. Not all of them would have been confirmed by culture, but from the isolates presented for phage typing it appears that at least a quarter of all notified cases had positive culture results. However, not all *S. typhi* isolates can be assumed to have been despatched for phage typing as this very much depends on the enthusiasm of staff in the different places.

During the study period 29 different phage types were encountered, plus the categories of 'untypable Vi-strains', 'degraded Vi-strains' and 'Vi negative strains'. The phage types found were A, B₁, B₂, C₁, C₄, C₁₀, D₁, D₂, D₈, D₉, D_{1-N}, E₁, E₂, E₄, E₇, F₁, F₃, F₄, G₁, G₂, J₁, J₅, K₁, L₁, M₁, M₂, N, 25, 53.

During the 10-year period as a whole the commonest phage type was A (20.9% of foci) followed by E₁ (14.8%), D₁ (10.3%) and degraded Vi positive strains (10.3%). The predominance of these strains is also evident when the results for each 2-year period of the study are examined separately.

Others which occurred relatively frequently were untypable Vi-strains (7.3%), C₄ (7.1%), D₂ (4.4%), E₂ (3.9%), 25 (2.6%), and B₁ (2.5%).

Phage types which were encountered every year were A, C₄, D₁, D₂, E₁, E₂, M₁, 25, degraded Vi-strains and untypable strains.

The phage types which appeared in the early years and then disappeared were B₂, D₉ and D_{1-N}, while others, including K₁ and 53 made their first appearance in recent years.

Types B₂, C₁, C₁₀, D_{1-N}, E₇, F₁, F₃, F₄, J₂, K₁, L₁, M₂ and 53 were encountered very infrequently.

Table 1. Frequency distribution of phage types of *S. typhi* for the 10 year period 1970-1979 (by cases)

(Figures in parentheses are percentages)

Years ...	1970-1	1972-3	1974-5	1976-7	1978-9	1970-9
Phage						
A	48 (21.2)	62 (18.5)	110 (28.0)	257 (29.2)	140 (21.3)	617 (24.1)
B ₁	0	39 (10.6)	6 (1.5)	5 (0.7)	0	50 (2.0)
B ₂	1 (0.5)	1 (0.4)	0	0	0	2 (0.1)
C ₁	0	0	0	1 (0.1)	3 (0.5)	4 (0.2)
C ₂	16 (6.6)	25 (7.5)	17 (4.4)	142 (13.0)	62 (9.3)	262 (10.2)
C ₁₀	0	0	0	1 (0.1)	0	1 (0.1)
D ₁	8 (3.6)	17 (5.2)	40 (10.2)	153 (15.9)	100 (15.2)	318 (12.4)
D ₂	9 (3.9)	8 (2.4)	10 (2.5)	22 (2.9)	42 (6.4)	91 (3.6)
D ₆	0	0	11 (2.8)	8 (1.1)	0	19 (0.7)
D ₉	20 (8.4)	24 (5.8)	0	0	0	44 (1.7)
D _{1-N}	0	3 (0.9)	0	0	0	3 (0.1)
E ₁	39 (16.9)	53 (14.6)	54 (13.8)	154 (16.9)	109 (16.4)	409 (15.9)
E ₂	20 (8.4)	11 (3)	21 (5.4)	20 (3.1)	14 (2.2)	86 (3.4)
E ₄	0	5 (1.2)	0	1 (0.1)	0	6 (0.2)
E ₇	0	0	0	1 (0.1)	0	1 (0.1)
F ₁	0	0	1 (0.3)	0	0	1 (0.1)
F ₃	0	0	0	1 (0.1)	0	1 (0.1)
F ₄	0	0	0	1 (0.1)	0	1 (0.1)
G ₁	3 (1.5)	7 (2.1)	2 (0.5)	8 (1.1)	0	25 (1.0)
G ₂	0	0	0	1 (0.1)	0	1 (0.1)
J ₁	0	2 (0.5)	10 (2.6)	6 (0.8)	34 (4.9)	52 (2.0)
J ₅	1 (0.5)	3 (0.9)	0	32 (2.9)	19 (3)	55 (2.2)
K ₁	0	0	0	0	1 (0.2)	1 (0.1)
L ₁	0	0	1 (0.3)	1 (0.1)	0	2 (0.1)
M ₁	4 (1.9)	9 (2.7)	3 (0.8)	3 (0.3)	10 (1.6)	29 (1.1)
M ₂	0	0	1 (0.3)	1 (0.1)	1 (0.2)	3 (0.1)
N	7 (3.9)	4 (1.2)	1 (0.3)	0	1 (0.2)	13 (0.5)
25	11 (5.5)	3 (0.9)	6 (1.5)	11 (1.2)	25 (3.8)	56 (2.2)
53	0	0	0	1 (0.1)	3 (0.5)	4 (0.2)
Untypable Vi strain	16 (6.6)	16 (4.8)	46 (11.8)	16 (1.5)	44 (6.5)	138 (5.3)
Degraded Vi +ve strain	26 (10.7)	59 (16.8)	41 (10.5)	47 (5.8)	41 (6.1)	214 (8.3)
Vi negative strain	0	0	10 (2.5)	28 (2.6)	6 (1.0)	44 (1.7)
Total	229	351	391	922	660	2553

Table 2. Frequency distribution of phage types of *S. typhi* for the 10 year period 1970-1979 (by foci)

(Figures in parentheses are percentages.)

Years ...	1970-1	1972-3	1974-5	1976-7	1978-9	1970-9
Phage type						
A	35 (20.5)	50 (17.3)	44 (16.8)	100 (25.5)	98 (22.0)	327 (20.9)
B ₁	0	29 (9.4)	6 (2.5)	5 (1.4)	0	40 (2.5)
B ₂	1 (0.6)	1 (0.4)	0	0	0	2 (0.1)
C ₁	0	0	0	1 (0.2)	2 (0.5)	3 (0.2)
C ₄	12 (6.5)	20 (7.3)	15 (5.8)	35 (8.8)	29 (6.5)	111 (7.1)
C ₁₀	0	0	0	1 (0.2)	0	1 (0.1)
D ₁	7 (4.1)	16 (5.6)	23 (8.6)	52 (13.1)	62 (14.0)	160 (10.3)
D ₂	6 (3.4)	8 (2.8)	10 (3.7)	15 (3.9)	29 (6.4)	68 (4.4)
D ₆	0	0	10 (3.7)	7 (2.1)	0	17 (1.1)
D ₉	18 (10.3)	17 (5.7)	0	0	0	35 (2.2)
D _{1-N}	0	3 (1.1)	0	0	0	3 (0.2)
E ₁	27 (15.4)	40 (13.4)	29 (11.3)	61 (15.9)	75 (16.6)	232 (14.8)
E ₂	14 (7.8)	10 (3.3)	16 (6.7)	9 (2.5)	12 (2.7)	61 (3.9)
E ₄	0	4 (1.3)	0	1 (0.2)	0	5 (0.3)
E ₇	0	0	0	1 (0.2)	0	1 (0.1)
F ₁	0	0	1 (0.5)	0	0	1 (0.1)
F ₃	0	0	0	1 (0.2)	0	1 (0.1)
F ₄	0	0	0	1 (0.2)	0	1 (0.1)
F ₄	0	0	0	1 (0.2)	0	1 (0.1)
G ₁	3 (1.8)	6 (2.1)	2 (0.7)	6 (1.7)	4 (0.9)	21 (1.3)
G ₂	0	0	0	1 (0.2)	0	1 (0.1)
J ₁	0	2 (0.6)	9 (3.7)	6 (1.6)	15 (3.2)	32 (2.1)
J ₅	1 (0.6)	3 (1.2)	0	21 (5.0)	13 (3.0)	38 (2.4)
K ₁	0	0	0	0	1 (0.2)	1 (0.1)
L ₁	0	0	1 (0.5)	1 (0.2)	0	2 (0.1)
M ₁	4 (2.4)	9 (3.2)	3 (1.2)	3 (0.8)	10 (2.3)	29 (1.9)
M ₂	0	0	1 (0.5)	1 (0.2)	1 (0.2)	3 (0.2)
N	7 (4.4)	4 (1.5)	1 (0.5)	0	1 (0.2)	13 (0.8)
25	7 (4.4)	2 (0.7)	6 (2.3)	11 (2.8)	15 (3.4)	41 (2.6)
53	0	0	0	1 (0.2)	3 (0.7)	4 (0.3)
Untypable Vi strain	15 (8.7)	16 (5.5)	35 (14.5)	13 (3.0)	34 (7.5)	113 (7.3)
Degraded Vi +ve strain	16 (9.1)	51 (17.5)	31 (12.8)	22 (6.0)	38 (8.3)	160 (10.3)
Vi negative strain	0	0	10 (3.7)	15 (3.9)	6 (1.4)	31 (2.0)
Total	173	291	253	393	448	1558

The geographical distribution of the phage types over the 10-year period shows the following interesting features.

There were very few strains submitted for typing from some states. This does not really represent the true picture of prevalence of typhoid in these states as the low numbers must reflect rather the facilities, enthusiasm and interest of staff in obtaining cultures and sending strains for phage typing.

Phage-type A was found in every state and E₁ in all but one.

Phage types which occurred in relatively small numbers naturally tended to be restricted in their distribution.

For instance, 16 out of 40 B₁ occurred in Negeri Sembilan, 3 out of 3 C₁ in Selangor, 10 out of 17 D₈ in Perak, 23 out of 35 D₉ in Selangor, 19 out of 32 J₁ in Kedah, 20 out of 38 J₅ in Penang and 8 out of 13 N in Selangor.

Type A was the most predominant phage type encountered in 7 of the 12 states which sent in strains while E₁ predominated in two states. C₁ and D₁ predominated in two other states respectively.

Type A which was the commonest phage type overall was found only in few numbers in Selangor and Kelantan although a relatively large number of isolates were typed from these states.

DISCUSSION

The 10-year study shows that in Malaysia the predominant strains encountered are A, E₁ and D₁. Others which occurred relatively frequently were degraded strains, untypable Vi-strains, C₄, D₂ and E₂.

While it was not exactly dramatic, variation in phage-type prevalence was noted among the different states. For instance types B₁, C₁, D₈, D₉, J₁, J₅ and N appeared to be relatively prevalent in certain states.

While there has been little change in the predominant strains over the 10-year period it was evident that strains which occurred in much smaller numbers were in some instances associated with some years. Some other countries have reported little change in the prevalence of the phage type over a number of years (French, King & St Louis, 1977).

Frequencies calculated from 'gross type distribution' – arrived at by including all cultures in the analysis – tends to vary from year to year, whereas net distribution, which is the analysis by foci of infection, is remarkably constant. This is understandable when it is realized that the ultimate source of all typhoid fever is the chronic carrier and the distribution of chronic carriers in the population changes only very slowly (Anderson & Williams, 1956).

Despite all the intermingling of strains of the typhoid bacillus caused by population migration, wars, etc., important differences in the geographical distribution of phage types are still evident. Some types are ubiquitous, while others appear more or less limited to one area or to a group of neighbouring areas. It seems that the typhoid bacillus, which was initially homogeneous, has diversified in various regions of the world and certain varieties, carried out of their original habitat, have reached distant regions while others have extended only to a limited degree from their original territory (International Committee for Enteric Phage Typing, 1973).

Looking at the global picture, type E₁ is the commonest, followed by A and D₁ and these are thus considered to be cosmopolitan types (Anderson & Williams, 1956). It is interesting that this situation has not changed much in over 20 years and in Malaysia today the three commonest types are also A, E₁ and D₁ in that order.

Some findings in other countries will now be described for comparison. In Japan, for the period 1956–74, the prevalent types were D₂, E₁ and M₁ (Ohashi, 1977). In Malaysia, while E₁ was second commonest, D₂ only constituted 4·4% while M₁ was 1·9%.

A 2-year study in Singapore, which is at the tip of the Malaysian Peninsula showed that the predominant phage types were B₁, D₁ and A accounting for 23%, 18% and 16% respectively (Lam & Goh, 1978). While D₁ and A were common in Malaysia, B₁ constituted only 2·5% while it was the most commonly occurring type in neighbouring Singapore. The state of Johore which is next to Singapore did not have any B₁ isolates over the 10-year period. However, the total number of isolates studied from Johore was rather small and this may explain why some B₁ cases may have been missed. Also, the 4th commonest type in Singapore was B₂ (8%) while in Malaysia it constituted only 0·1%.

Studies in Jamaica showed that E₁ constituted 60% of all strains typed while the next commonest was phage type 45 which is almost unique to Jamaica (French, King & St. Louis, 1977).

A series in Madurai, India (Uma *et al.* 1980) indicated that A and E₁ accounted for 78·9% of all isolates while the third-commonest, K₁, constituted only 2·1%. There was only one strain of K₁ isolated in Malaysia over the 10-year period.

These differences emphasize the need for phage-type patterns to be studied and point to the usefulness of the knowledge of such patterns in efforts to control the disease.

The author wishes to record his appreciation to Dr R. Bhagwan Singh, who initiated the phage-typing scheme in Malaysia, also all the persons who sent in strains for phage typing, and the following for technical assistance during the 10-year period – N. Manokaran, Ng Poh Loon, M. Kanaganayagy and Cheah Chin Tiew and Thean Yit Sang for typing the manuscript. The author also wishes to thank the Director, Institute for Medical Research, Kuala Lumpur, for his kind permission to publish this paper.

REFERENCES

- ANDERSON, E. S. & WILLIAMS, R. E. O. (1956). Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *Journal of Clinical Pathology* **9**, 94–127.
- CRAIGIE, J. & YEN, C. H. (1938). Demonstration of types of *B. typhosus* by means of preparations of type II Vi phage: principles and techniques. *Canadian Public Health Journal* **29**, 448–463.
- FRENCH, G. L., KING, S. D. & ST LOUIS, P. (1977). *Salmonella* serotypes, *Salmonella typhi* phage types and antimicrobial resistance at the University Hospital of the West Indies, Jamaica. *Journal of Hygiene* **79**, 5–16.
- FURUMI, H. (1977). Intelligence activity and its significance in disease control. *Proceedings of the 3rd SEAMIC Seminar, 17–28 February 1976, Tokyo, Japan*, pp. 3–11.
- INTERNATIONAL COMMITTEE FOR ENTERIC PHAGE TYPING. (1973). The geographical distribution of *Salmonella typhi* and *Salmonella paratyphi A* and *B* phage types during the period 1 January 1966 to 31 December 1969. *Journal of Hygiene* **71**, 59–84.

- LAM, S. Y. S. & GOH, K. T. (1978). Vi phage types in Singapore. *Singapore Medical Journal* **19**, 3-8.
- LO, E. (1977). *Typhoid in Peninsula Malaysia*. Ministry of Health Report (unpublished).
- OHASHI, M. (1977). Phage type distribution and antibiotic resistance in *S. typhi*, *S. paratyphi A* and *S. paratyphi B* isolated in Japan in 1956-1974. *Proceedings of the 3rd SEAMIC Seminar, 17-28 February 1976, Tokyo, Japan* pp. 32-38.
- SINGH, R. B. (1971). Division of Bacteriology. *Annual Report of the Institute for Medical Research, Kuala Lumpur, Malaysia*, pp. 25-46.
- UMA, A., SHARMA, K. B., THIRUMALAIKOLUNDUSUBRAMANIAM, P., MUTHU, A. K. & THIGARAJAN, R. (1980). Prevalence of *Salmonella* phage types in Maduræ. *Indian Journal of Medical Research* **71**, 171-174.