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Comparative Analysis of *Salmonella typhi* by rRNA Gene Restriction and Phage Typing

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Abstract: Phage typing and ribotyping were used to analyze 19 isolates of *Salmonella typhi* from sporadic cases and two outbreaks of typhoid fever in Malaysia in 1987 and 1990. The two outbreaks were associated with phage types D1 and E1 in Penang and Alor Setar respectively, and phage types D1, E1 and A (with 3 untypeable isolates) were present among the sporadic cases. Ribotyping detected 14 ribotypes among the 19 isolates thus establishing its higher discriminative capacity compared to phage typing. The outbreak isolates were more homogeneous by ribotyping and the data also suggested that one outbreak was propagated, multi-focal in nature (Penang) while another was a point- source traceable to a single event (Alor Setar).

Keywords: *Salmonella typhi*, ribotyping, phage typing

Introduction

Typhoid fever, the enteric fever caused by the bacterium *Salmonella typhi*, continues to pose an important public health problem in many developing countries including Malaysia. The endemicity of typhoid fever in the developing countries of the Third World has been exacerbated by antibiotic-resistance, increased incidence among HIV-infected individuals, and the large scale movement of migrant workers from high incidence regions. Monitoring the spread and movement *S. typhi* strains will require an effective surveillance system based on the ability to discriminate individual isolates. Classical methods of strain analysis, including phage typing, serotyping and plasmid profiles have their limitations with regards to practicability and discriminating capacity, and there has thus been an increasing interest in molecular typing methods, including pulsed-field gel electrophoresis, *IS*200 profiling, and ribotyping. Although the value and sensitivity of ribotyping or rDNA gene restriction patterns analysis has been shown in several previous studies (Pang *et al.*, 1992; Fica *et al.*, 1996), there is a need to apply and evaluate this method more extensively with more strains in different endemic regions. More importantly, information on molecular types collected from various typhoid-endemic areas may eventually form the basis of an epidemiological surveillance system. We report here the use of ribotyping to analyze isolates of *S. typhi* obtained from outbreak and sporadic cases of typhoid fever in Malaysia in 1987-1990.

Materials and Methods

Bacterial isolates: Isolates of *S. typhi* from either the blood or stools of humans were used in the present study. The organisms were isolated, maintained and identified by standard procedures. Ten isolates were obtained from two well-documented outbreaks in northern parts of Malaysia between 1987 and 1990. In addition, nine isolates from sporadic cases of typhoid fever were obtained during the same time period and in approximately the same geographical locations. Vi phage typing of the isolates was performed by standard procedures by the Salmonella Reference Centre at the Institute for Medical Research, Kuala Lumpur. Susceptibility to antibiotics was determined by standard disk-diffusion procedures (NCCLS, 1995). The plasmid content was also determined by the alkaline lysis method of Birnboim and Doly (1979).

Ribosomal gene restriction analysis: Ribotyping (rDNA typing) of these isolates was performed as previously described Altwegg *et al*. (1989). Briefly, genomic DNA was digested with restriction endonucleases, *Pst*I and *Sma*I (New England Biolabs, Beverly, MA, USA), electrophoresed on 1% agarose gel in Tris-acetate buffer (0.04M Tris acetate, 0.02M EDTA) and stained in ethidium bromide (1 µg/mg) for 20 minutes. Gels were then photographed and blotted onto a nitrocellulose membrane according to standard procedure (Maniatis *et al*., 1982). The membrane was then probed with plasmid pKK3535 (containing one copy each of the genes coding for 5S, 16S, 23S and tRNA from *E. coli*) as described previously (Altwegg *et al.*, 1989). Each ribotype was assigned an arbitrary pattern type and compared by calculating a Dice or similarity coefficient using the following formula: $F = 2n_{xy}/(n_x + n_y)$, where n_x is the total number of DNA bands from isolate X, n_y is the total number of DNA bands from isolate Y, and n_{xy} is the total number of DNA bands that were identical in the two isolates. Isolates were considered to be genetically similar or identical if there was complete concordance of the DNA band profiles and were considered different, for the purpose of pattern comparison only, if there was a difference of one or more DNA bands. Using this method, an F value of 1.0 indicates identical patterns and an F value of 0 suggests complete dissimilarity.

Results and Discussion

A total of 19 human isolates of *S. typhi* from either blood or feces were used in this study: 5 isolates were obtained during an outbreak on Penang Island in 1987, 5 from Alor Setar outbreak in 1990 and 9 were isolates from sporadic cases of typhoid fever during the same time period. All the isolates were sensitive to ampicillin, chloramphenicol, kanamycin, streptomycin, spectinomycin, trimethoprim, sulfonamides, gentamicin, neomycin, carbenicillin, cephalothin, nalidixic acid, cephalothin, co-trimoxazole and tetracycline. No plasmid was detected.

Among the 19 isolates studied, three different phage types were detected. Isolates from the Penang outbreak were phage type D1, those from the Alor Setar outbreak were phage type E1 and the sporadic isolates consisted of phage types D1 (4), A (1), E1 (1) and 3 isolates were untypeable (Table 1), After digestion with *Sma*I (Fig. 1A), a single rDNA pattern was detected among the 5 isolates from the Alor Setar outbreak (pattern B) and two, very similar pattern (A and A′, F = 0.9) were present among the 5 isolates from the Penang outbreak (Table 1). In contrast, digestion with *Pst*I (Fig. 1B) produced two (E and E′, F = 0.83) and four patterns (A, B, C, D: F = 0.67-0.92) among the Alor Setar and Penang outbreak isolates respectively (Table 1). Five different patterns were seen among the 9 sporadic isolates (A, A′, B, B′, C) with *Sma*I and seven patterns (F, G, H, B, I, J, L) with *Pst*I (Table 1). Of these seven patterns associated with *Pst*I except for three isolates with pattern K, each of the isolates was associated with a unique ribotype (F = 0.3-0.91). Overall, 14 different ribotypes were detected among the 19 isolates analyzed (Table 1). One of the sporadic isolates (#281) showed identical banding patterns to one of the outbreak (#1054) by both restriction endonucleases (combined ribotype 2) (Table 1). There was no correlation observed between phage types and ribotypes.

Ribotyping has been applied to both sporadic and outbreak of *S. typhi* strains from Italy (Nastasl *et al*., 1991), Chile (Fica *et al.,* 1996) and Spain (Navarro *et al.,* 1996). However, there is a clear paucity of ribotyping data from strains isolated in Southeast Asia, one of the major endemic regions. Given the genetic diversity of

Table 1: Ribotypes and Phage types of Malaysian isolates of *S. typhi*

Isolate	Place and date of isolation type	Phage type	rDNA gene restriction patterns fater digestion with[+] *Sma*I	*Pst*I	Combined Ribotype
1010	Penang (1987)	D1	A	A	1
1054	Penang (1987)	D1	A′	B	2
1106	Penang (1987)	D1	A	C	3
1023	Penang (1987)	D1	A′	D	4
1066	Penang (1987)	D1	A′	D	4
259	Alor Setar (1990)	E1	B	E′	5
253	Alor Setar (1990)	E1	B	E	6
252	Alor Setar (1990)	E1	B	E	6
263	Alor Setar (1990)	E1	B	E	6
245	Alor Setar (1990)	E1	B	E	6
282	Johor (1990)	D1	A	F	7
495	Muar (1987)	D1	A′	G	8
436	Alor Setar (1990)	Unt[#]	B	H	9
281	P.Besar (1990)	D1	A′	B	2
850	B.Mertajam (1987)	D1	A	I	10
13	Muar (1987)	Unt[#]	A′	J	11
915	Bentong (1987)	A	C	K	12
21	Ipoh (1987)	E1	B′	K	13
7	Kulim (1987)	Unt[#]	B	K	14

*Penang and Alor Setar isolates were from outbreaks of typhoid fever. The last nine isolates listed were from sporadic cases of typhoid fever. [+] Patterns A/A′ and B/B′ (*Sma*I) and E/E′) (*Pst*I) were very closely related and differed by a slight shift in the position of a single band. [#] Unt = untypeable

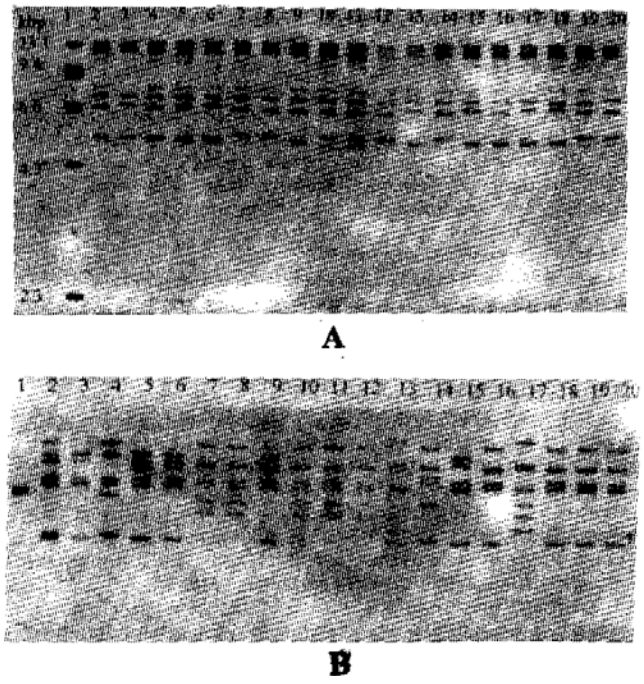

Fig. 1: rDNA gene restriction patterns of *S. typhi* isolates from outbreak and sporadic cases of typhoid fever following digestion with *Sma*I (A) and *Pst*I (B) and hybridization with pKK3535. Lane 1 = Lambda *Hin*dIII digest, with marker size indicated in kbp. Lanes 2-6: Isolates from the Penang outbreak; Lanes 7-8, 10-12: Isolates from the Alor Star outbreak; Lanes 9,13-20: sporadic isolates (note: the sequence of the lane numbering corresponds to the strain number in Table 1, except lane 9 = #282)

S. typhi strains (Thong *et al.*, 1994), it would seem important to collect such data. We have previously analysed 20 Malaysian isolates of *S. typhi* from sporadic cases by ribotyping (Pang *et al.*, 1992) and have now extended our study to strains isolated during two well-defined outbreaks of typhoid fever in Peninsular Malaysia. Several conclusions can be made from the present study. It is clear that the discriminative capacity of ribotyping is influenced by restriction endonuclease used; the present study confirms the earlier findings that *Pst*I was more discriminative than *Sma*I (Pang *et al.*, 1992). Isolates from outbreaks are more homogeneous and closely related compared to sporadic isolates. Also, outbreaks of typhoid fever can be caused by different ribotypes and certain outbreaks (e.g. Alor Setar) are caused by a single ribotype whereas others (e.g. Penang) can involve several different ribotypes. These observations are in agreement with the available epidemiological information which suggested that the Penang outbreak in 1987 was a large, propagated, multifocal outbreak related to food contaminated by carriers, whereas the Alor Setar outbreak in 1990 was a more localised, point-source outbreak occurring in a village after a wedding feast. From the number of different ribotypes identified, it can also be concluded that multiple, independent clones of *S. typhi* co-exist simultaneously, causing sporadic cases of typhoid fever among the population throughout the year. Similar conclusions have been made when *S. typhi* strains were analyzed by pulsed-field gel electrophoresis (Thong *et al.*, 1994).

Conclusion

The present study confirms the usefulness of ribotyping as a highly discriminative technique for the differentiation and typing of epidemiologically-defined *S. typhi* isolates. The use of ribotyping, as a complement to classical typing methods, should be considered in future attempts at epidemiological surveillance of this important human patogen.

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