Journal of Clinical Microbiology, Nov. 1996, p. 2831–2834 Vol. 34, No. 11
0095-1137/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Molecular Typing of *Salmonella enterica* Serovar Typhi

F. NAVARRO,[1,2]* T. LLOVET,[1,2] M. A. ECHEITA,[3] P. COLL,[1,2] A. ALADUEÑA,[3] M. A. USERA,[3] AND G. PRATS[1,2]

Servei de Microbiologia, Hospital de Sant Pau, 08025 Barcelona,[1] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra,[2] and Sección de Enterobacterias, Servicio de Bacteriología, Centro Nacional de Microbiología, Virología e Inmunología Sanitarias, Instituto de Salud Carlos III, 28220 Majadahonda,[3] Spain

Received 4 April 1996/Returned for modification 21 June 1996/Accepted 2 August 1996

The efficiencies of different tests for epidemiological markers—phage typing, ribotyping, IS*200* typing, and pulsed-field gel electrophoresis (PFGE)—were evaluated for strains from sporadic cases of typhoid fever and a well-defined outbreak. Ribotyping and PFGE proved to be the most discriminating. Both detected two different patterns among outbreak-associated strains.

Traditional characterization of *Salmonella enterica* serovar Typhi depends on phage typing; however, this method is not very discriminating (8). In the last few years, epidemiological investigations have been substantially improved by the application of molecular techniques (1, 4, 5, 11–13, 17, 18).

In the present study, we evaluated the efficiencies of different tests for epidemiological markers—phage typing, ribotyping, IS*200* typing, and pulsed-field gel electrophoresis (PFGE)—for the differentiation of individual isolates of *S. enterica* serovar Typhi and of isolates involved in a large, well-defined outbreak of typhoid fever.

A total of 83 *S. enterica* serovar Typhi isolates, 48 from sporadic cases and 35 from a large outbreak, were selected for inclusion in this study. The 48 sporadic-case strains belong to the collection of the Spanish National Reference Laboratory for *Salmonella*. They were isolated in different geographical locations, and nonepidemiological relationships among them were documented. The 35 outbreak isolates were from 25 patients and the food handler from an unusually prolonged outbreak of typhoid fever originating from a casual food handler who was an asymptomatic carrier.

Phage typing was performed with the phage set provided by the WHO International Phage Typing Laboratory for *Salmonella* at Colindale, London, United Kingdom (7).

Genomic DNA was prepared by treatment with hexadecyltrimethylammonium bromide according to *Current Protocols in Molecular Biology* (2). DNA fragments were digested with *Hin*dIII and were analyzed by Southern blotting. Transferred DNA fragments were hybridized with digoxigenin-labeled *Escherichia coli* rRNA (Sigma, St. Louis, Mo.) or with an IS*200* probe generated by nested PCR (6).

DNA for PFGE analysis was prepared by the method of Smith et al. (16). The restriction endonuclease *Xba*I was used on *S. enterica* serovar Typhi outbreak strains and also on isolates from sporadic cases. Outbreak strains were also digested with *Xho*I. PFGE was performed by orthogonal-field-alternation electrophoresis (Gene Navigator; Pharmacia, Uppsala, Sweden). Macrorestriction fingerprints were compared by using the Bio Image system (Millipore, Bedford, Mass.). The similarities of the fragment length patterns of two strains were scored by the Dice coefficient (14). Strains were clustered by the method of unweighted pair-group averages (10). The banding patterns were interpreted according to the guidelines of Bannerman et al. (3).

The discriminatory abilities of the different tests were calculated by the index of Hunter and Gaston (9).

Table 1 summarizes the results of the different tests employed. Eleven different phage types were detected among 48 sporadic-case strains, and 8 sporadic-case strains shared the same phage type with the outbreak strains. Although phage typing is the classical epidemiological test for *S. enterica* serovar Typhi, it is technically difficult and can be performed only by reference laboratories. Moreover, it can give low discriminatory power when strains isolated from local cases, in which a small number of phage types are usually predominant, are studied. In our case the discriminatory index was 0.890.

Only eight different profiles were obtained with IS*200*. Forty-one sporadic-case strains and all 35 outbreak strains belonged to type 1. The low discriminatory index (0.273) suggests, according to previous studies (18), that this epidemiological test should not be used as a typing method for this serotype.

Thirty-one different ribotypes were observed in the sporadic-case strains (Fig. 1). The discriminatory index was 0.974. Two different patterns were detected in the 35 outbreak strains. Twenty-four strains showed an identical pattern (type 24). Eleven strains had a different but closely related pattern (type 25). Several strains of different samples from seven outbreak patients, including the food handler, were studied, with the following results: five patients had only one ribotype (four had ribotype 24 and one had ribotype 25), and two patients, including the food handler, had both ribotypes. Two strains from sporadic cases showed the same patterns as the outbreak strains.

Thirty-eight different PFGE patterns were observed in the 48 sporadic-case strains after digestion with *Xba*I, with a range of similarity between 35 and 100% (Fig. 2 and 3). The discriminatory index was 0.988. One of these strains showed a pattern identical to that of the outbreak strains. This strain could be differentiated from the outbreak strains only by ribotyping. Three sporadic-case strains were considered to constitute a subtype of the outbreak strain, as shown by their digestion pattern.

The 35 isolates from the outbreak appeared to be genetically identical when digested with *Xba*I. In contrast, these strains showed two different digestion patterns when *Xho*I was used. These patterns differed by two bands (data not shown). Each

* Corresponding author. Mailing address: Servei de Microbiologia, Hospital de Sant Pau, Avda Sant Antoni Mª Claret, 167, 08025 Barcelona, Spain. Phone: 34 3 2919071. Fax: 34 3 2919070.

TABLE 1. Phage types, ribotypes, IS*200* types, and PFGE patterns of sporadic strains

Strain	Phage type	Ribotype	IS*200* type	PFGE pattern
1	I+IV	17	1	11
5	46	15	5	1c
6	D6	19	1	22
7	46	20	1	1d
8	E1a	21	1	25
9	C1	22	1	5
13	46	26	6	17
15	C1	27	1	20
16	B2	24	1	21
19	46	27	1	1a
22	B2	25	1	14a
24	Ade	2	1	14b
25	A	10	1	16
35	A	18	7	2
36	A	29	1	27
39	F1	30	1	19
40	B2	31	1	12b
43	C1	2	1	28
48	I+IV	31	1	9
49	I+IV	33	9	3
50	B2	18	1	4
52	E1a	34	1	8
55	46	19	1	1b
60	A	1	2	29a
61	C1	2	1	30a
62	D1	3	1	31
63	E1a	1	1	40a
64	46	4	1	29b
65	Ade	2	1	38
66	A	5	1	30b
67	E1a	4	1	40b
68	34	6	1	33
69	Ade	7	1	34
70	46	8	1	26
71	46	9	1	7
72	Ade	10	1	10
73	E1a	11	1	41
74	Ade	10	1	39
75	C1	12	3	18
76	B2	13	1	32
77	Ade	1	1	36
78	Ade	12	1	38
79	E1a	14	1	42
80	C1	15	1	37
81	A	1	1	35
82	I+IV	16	4	12a
83	E1a	1	1	40c
84	Ade	10	1	13

pattern corresponds to one ribotype. Results for strains from the same patient were identical to the results obtained by ribotyping.

Evidence that individual humans may be infected simultaneously by strains of different clones has been documented previously in countries with a high incidence of typhoid fever (15). The possibility of two different outbreaks was disregarded because of the epidemiological relationship among isolates from outbreak-related patients and the detection of both patterns for the food handler. The selection by subculture of a single colony could explain the fact that only one pattern was detected in the majority of patients. These data suggest a double infection in the food handler. Nevertheless, the homology detected in these strains, the high heterogeneity detected among the nonrelated strains, the long time of asymptomatic carriage of the food handler, and the low incidence of typhoid fever in Spain (1.9/100,000 in 1993) (19) suggest that one of the isolates in the food handler may be derived from the other.

In summary, we found a high correlation between ribotyping and PFGE with regard to the ability to differentiate and group *S. enterica* serovar Typhi isolates. Considerable heterogeneity at the DNA level among *S. enterica* serovar Typhi isolates has been detected by both methods. Extreme caution should be exercised in the analysis of ribotyping results, since a single genetic change can originate changes in two or three bands; because of the low number of bands generated in ribotyping, this could affect 50% of the pattern, and closely related strains would fall apart in the cluster analysis. PFGE, generating a large number of bands (between 15 and 21 in our study), allows a more accurate cluster analysis when variations of two or three bands occur, giving a high similarity index among closely related strains. This test is thus the most appropriate for outbreak analysis. On the other hand, compared with ribotyping, PFGE is easier to perform because it does not involve the transfer of DNA fragments from agarose gels to solid supports or subsequent hybridization.

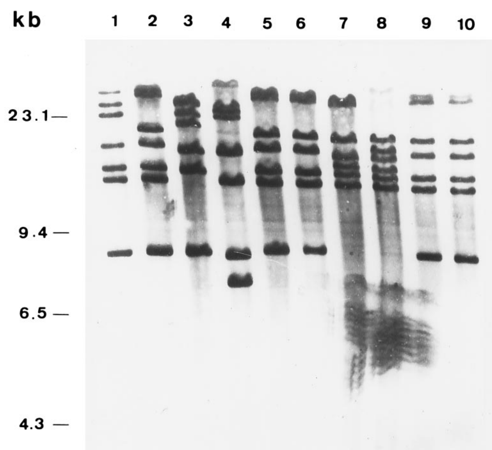

FIG. 1. Southern hybridization analysis of genomic DNA from *S. enterica* serovar Typhi digested with *Hin*dIII and probed by the rRNA gene. Lanes 1, 2, 5, 6, 9, and 10, outbreak strains (ribotype 25 in lane 1 and ribotype 24 in the others); lanes 3, 4, 7, and 8, sporadic-case strains (ribotypes 30, 31, 2, and 12, respectively).

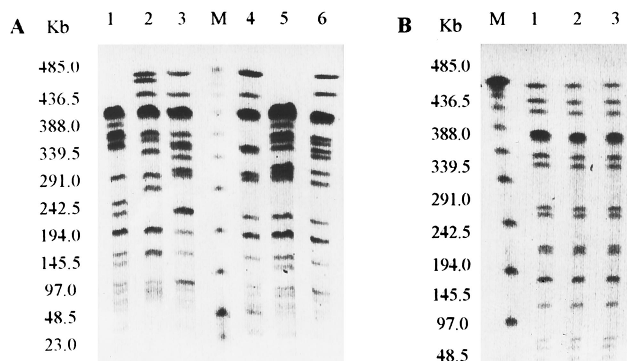

FIG. 2. Agarose gel showing the *Xba*I digestion patterns of *S. enterica* serovar Typhi isolates involved in sporadic cases of typhoid fever (A) and in an outbreak from Terrassa (B). M, DNA size standards (lambda DNA concatemers; New England Biolabs, Beverly, Mass.).

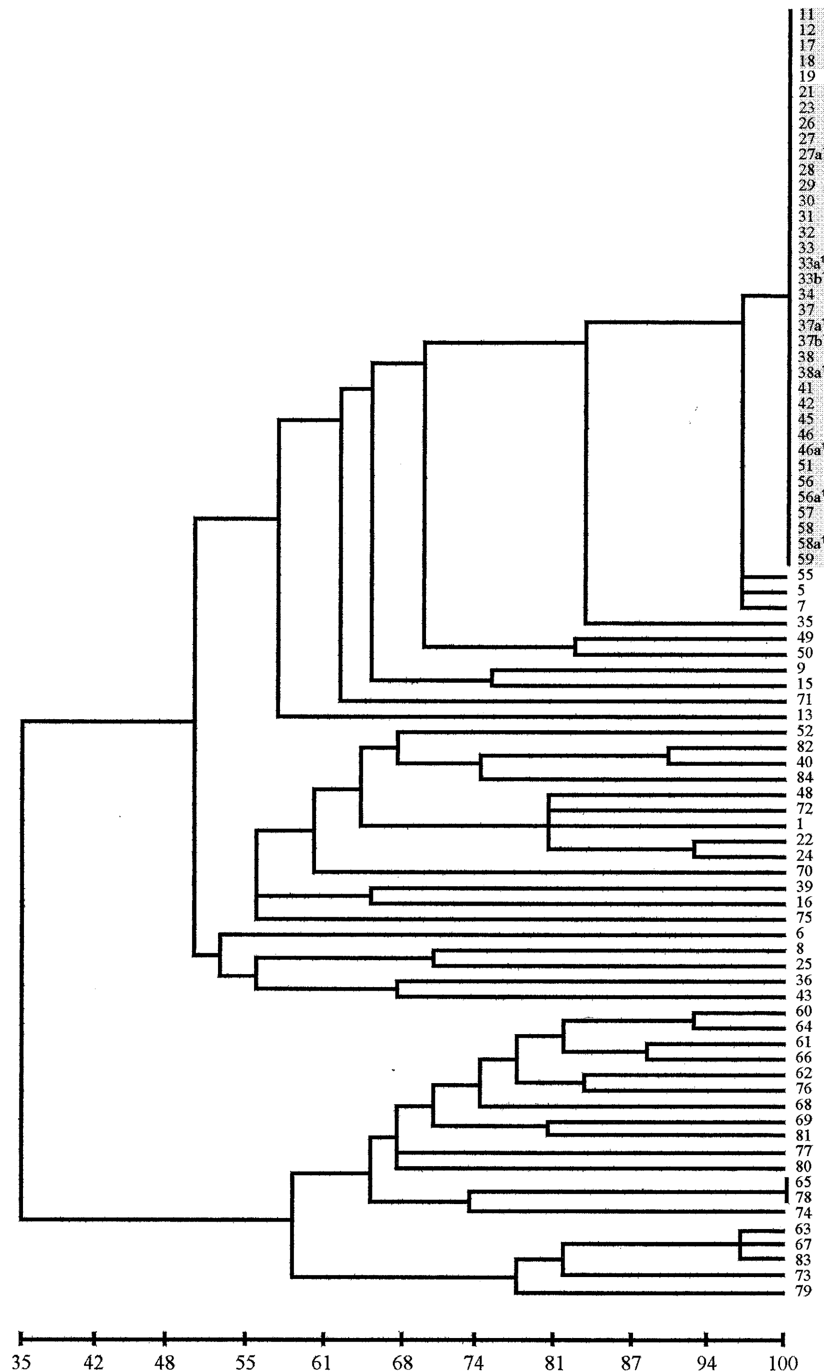

FIG. 3. Dendrogram showing similarities among the *Xba*I pulsotypes of the 83 *S. enterica* serovar Typhi strains, including 48 sporadic-case strains and 35 strains involved in an outbreak. Shaded numbers are designations of outbreak strains. [1], different strains from the same patients.

We thank M. Xercavins, J. Moré, and N. Freixes from Hospital mutua de Terrassa and M. A. Morera, M. Simó, and F. Bella from Hospital de Terrassa for the gift of strains and for data collection.

REFERENCES

1. **Altwegg, M., F. W. Hickman-Brenner, and J. J. Farmer III.** 1989. Ribosomal RNA gene restriction patterns provide increased sensitivity for typing *Salmonella Typhi* strains. J. Infect. Dis. **160:**145–149.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1991. Current protocols in molecular biology, supplement 13, p. 2.4.1–2.4.5. Greene Publishing Associates and Wiley Interscience, New York.
3. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
4. **Baquar, N., A. Burnens, and J. Stanley.** 1994. Comparative evaluation of molecular typing of strains from a national epidemic due to *Salmonella brandenburg* by rRNA gene and IS*200* probes and pulsed-field gel electrophoresis. J. Clin. Microbiol. **32:**1876–1880.
5. **Franco, A., C. Gonzalez, O. S. Levine, R. Lagos, R. H. Hall, S. L. Hoffman, M. A. Moechtar, E. Gotuzzo, M. M. Levine, D. M. Hone, and J. G. Morris, Jr.** 1992. Further consideration of the clonal nature of *Salmonella typhi*: evaluation of molecular and clinical characteristics of strains from Indonesia and Peru. J. Clin. Microbiol. **30:**2187–2190.

6. **Gibert, L., K. Carroll, D. Hillyard, J. Barbé, and J. Casadesúa.** 1991. IS*200* is not a member of the IS*600* family of insertion sequences. Nucleic Acids Res. **19:**1343.
7. **Guinnée, P. A. M., and W. J. Van Neuween.** 1978. Phagetyping of *Salmonella*. Methods Microbiol. **11:**157–191.
8. **Hickman-Brenner, F. W., J. J. Farmer III, and Regional Centers for *Salmonella typhi* Bacteriophage Typing in the United States.** 1983. Bacteriophage types of *Salmonella typhi* in the United States from 1974 through 1981. J. Clin. Microbiol. **17:**172–174.
9. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
10. **Li, W. H.** 1981. Simple method for constructing phylogenetic trees from distance matrices. Proc. Natl. Acad. Sci. USA **78:**1085–1089.
11. **Nair, S., C. L. Poh, Y. S. Lim, L. Tay, and K. T. Goh.** 1994. Genome fingerprinting of *Salmonella typhi* by pulsed-field gel electrophoresis for subtyping common phage types. Epidemiol. Infect. **113:**391–402.
12. **Nastasi, A., C. Mammina, and M. R. Villafrate.** 1991. rDNA fingerprinting as a tool in epidemiological analysis of *Salmonella typhi* infections. Epidemiol. Infect. **107:**565–576.
13. **Reeves, M. W., G. M. Evins, A. A. Heiba, B. D. Plikaytis, and J. J. Farmer III.** 1989. Clonal nature of *Salmonella typhi* and its genetic relatedness to other salmonellae as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori* comb. nov. J. Clin. Microbiol. **27:**313–320.
14. **Römling, U., D. Grothues, T. Heuer, and B. Tümmler.** 1992. Physical genome analysis of bacteria. Electrophoresis **13:**626–631.
15. **Selander, R. K., P. Beltran, N. H. Smith, R. Helmuth, F. A. Rubin, D. J. Kopecko, K. Ferris, B. D. Tall, A. Cravioto, and J. M. Musser.** 1990. Evolutionary genetic relationships of clones of *Salmonella* serovars that cause human typhoid and other enteric fevers. Infect. Immun. **58:**2262–2275.
16. **Smith, C. L., S. R. Klco, and C. R. Cantor.** 1988. Pulsed-field gel electrophoresis and the technology of large DNA molecules, p. 41–72. *In* K. Davies (ed.), Genome analysis: a practical approach. IRL Press, Oxford.
17. **Thong, K.-L., Y.-M. Cheong, S. Puthucheary, C.-L. Koh, and T. Pang.** 1994. Epidemiologic analysis of sporadic *Salmonella typhi* isolates and those from outbreaks by pulsed-field gel electrophoresis. J. Clin. Microbiol. **32:**1135–1141.
18. **Threlfall, E. J., E. Torre, L. R. Ward, A. Dávalos-Pérez, B. Rowe, and I. Gibert.** 1994. Insertion sequence IS*200* fingerprinting of *Salmonella typhi*: an assessment of epidemiological applicability. Epidemiol. Infect. **112:**253–261.
19. **Usera, M. A., A. Echeita, A. Aladueña, J. Alvarez, C. Carreño, A. Orcau, and C. Planes.** 1995. Investigación de un brote de fiebre tifoidea de origen hídrico ocurrido en Cataluña en 1994. Enferm. Infecc. Microbiol. Clin. **13:**450–454.