Study of genetic diversity of *Salmonella typhi* using pulsed-field gel electrophoresis

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Abstrak

Telah dilakukan analisis kekerabatan genetik isolat Salmonella typhi yang di isolasi dari pasien demam tifoid sporadik yang dirawat inap di RS Persahabatan, Jakarta, pada semester I tahun 1998. Dari 25 isolat yang dicerna dengan XbaI ditemukan pola elektroforesis medan listrik berpulsasi (PFGE) yang bervariasi dan diidentifikasi 18 tipe PFGE. Analisis sidik gerombol menunjukkan bahwa kesemua isolat S. typhi berasal dari dua grup utama, dan pada tingkat kesamaan ≥ 84% ditemukan 7 sidik gerombol. Penelitian ini menunjukan adanya keragaman genetik yang cukup tinggi pada berbagai isolat S. typhi, dan tampaknya berbagai isolat ini berasal usul bukan dari klon tunggal. ***(Med J Indones 2001; 10: 158-63)***

Abstract

DNA genomes of Salmonella typhi (S.typhi), which were isolated from sporadic typhoid fever cases who were hospitalized in Persahabatan Hospital, Jakarta, during the 1st semester of 1998, were examined for their genetic diversities. Pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with XbaI was performed for 25 isolates. Electrophoresis patterns of most isolates varied and 18 PFGE types were identified. Cluster analysis showed that all isolates originated from two main groups; and at ≥ 84% level of similarity, 7 clusters were found. Thus, the results showed that genetic diversity of S. typhi was considerably high, and that S. typhi isolated from sporadic typhoid fever cases were derived from multiple clones. ***(Med J Indones 2001; 10: 158-63)***

Keywords: typhoid fever, sporadic cases, XbaI, cluster analysis

Intensive studies of genetic diversity and molecular epidemiology of *S.typhi,* the etiologic agent of typhoid fever, have been carried out since early nineties. Quite a range of *S.typhi* isolates for various geographic regions representing sporadic cases, outbreaks, and environmental isolates have been analysed using pulse-field gel electrophoresis (PFGE). Those studies showed that there were significant diversity among *S.typhi* isolates, in which isolates originated from outbreak cases were more homogenous compared to sporadic isolates. Also, it is suggested that there was a movement of certain strains within Southeast Asia.[1-5] In Indonesia, the bacteria causes a disease with a broader spectrum of serious complications, among others, typhoid hepatitis and typhoid pancreatitis.[6] Further, mortality is frequently seen in Indonesia, while in other part of Asia, the disease manifestations are milder. Previous study by Thong et al, 1996[7] reported that certain PFGE types were associated with the ability to cause a fatal disease.

In the present study, we applied PFGE technique to analyse large fragments of chromosomal DNA to enable us to determine the genetic relationship among *S.typhi* isolates. The result obtained can be used for epidemiology purposes, since it can contribute in the construction of highly accurate *S. typhi* genetic map for the whole region in Indonesia.

METHODS

All procedures below were performed in the Laboratory of the Department of Microbiology, Faculty of Medicine, University of Indonesia, during 1999-2000, except for the electrophoresis using CHEF DR III electrophoresis unit, which were conducted in Microbes and Genetic Engineering Division, R&D Centre for Biotechnology, Indonesia Institute of Science, Cibinong.

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Bacteria

Twenty five *S.typhi* isolates were randomly chosen from the collections of Dr. RHH Nelwan, Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Jakarta. The isolates were originated from sporadic cases of typhoid fever, which were hospitalized in the first semester of 1998, in Persahabatan Hospital, Jakarta. All isolates were tested for antibiotic susceptibility by standard disk diffusion procedures. No resistance was observed to amoxicillin (AML), amoxicillin clavunamic acid (AMC), chloramphenicol (C), trimethoprim sulpha-methoxazole (SXT), ciproxin (CIP), ofloxacin (OFP), lefofloxacin (LFX), streptomycin (S), tetracyclin (T), cefdinir (CFN), cefprozil (CPR), ceftriaxone (CRO), cefepime (FEP), or amikacin (AN).

PFGE and genome analysis

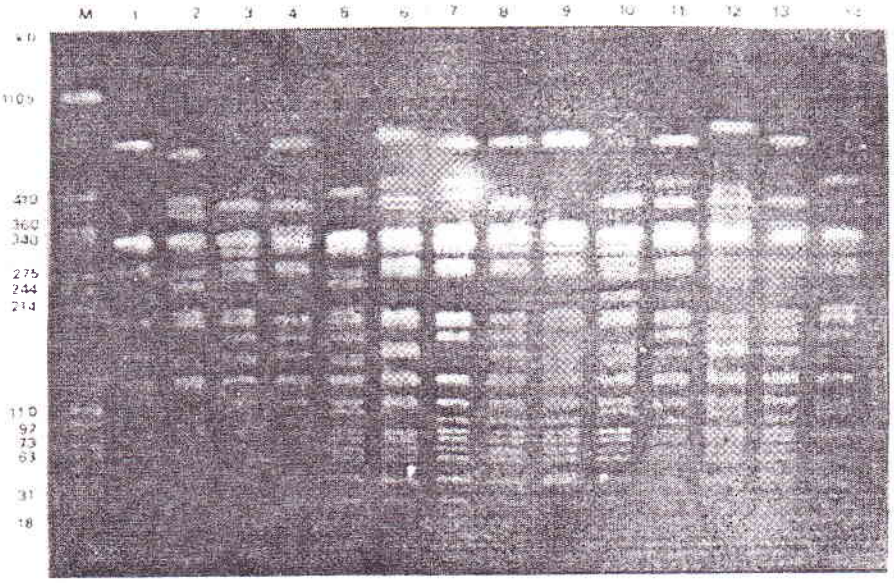
Chromosomal DNA was prepared as described by Suwanto and Kaplan, 1989,[8] and Thong et al, 1994.[5] In brief, overnight cultures were centrifuged at 4000g for 5 min. at 4°C and washed twice in 1 ml buffer containing 10 mM Tris HCl pH 7.5 and 1 M NaCl. Pellets were resuspended in the same buffer and mixed with 1% low melting agarose in 1x TE buffer (10 mM Tris HCl pH 8.0 and 1 mM EDTA pH 8.0). The agarose blocks were then subjected to lysing solution containing 1 mg/ml lysozyme and incubated at 37°C overnight. The following day, the lysing solution were replaced with solution containing 100 µg/ml proteinase K, 0.5 M EDTA pH 8.0 and 10% sarcosyl followed by incubation at 55°C for 48 hours. The *Xba*I restriction enzyme (Promega, Madison, WI, USA) was used, and digestion was performed according to the manufacturer. The digested chromosomal DNA were then electrophoresed using CHEF DR III electrophoresis unit (Biorad Laboratories, Hercules, CA, USA) at 14°C with a 120° switch angle, and a running time of 20 hours with a linear ramp of switching time from 5–50 sec, at 6 Volt/cm^2. *Rhodobacter sphaeroides* 2.4.1 digested with *Ase*I[8] was generously provided by DR. Antonius Suwanto from Inter University Center for Biotechnology, Institute of Agriculture Bogor, Bogor, Indonesia, and used as molecular marker. Gels were stained with ethidium bromide, destained and photographed under UV light.

Interpretation of PFGE gel patterns was carried out as described by Bannerman et al, 1995,[9] and Zadoks et al, 2000,[10] and assigned for arbitrary pattern types, and compared by calculating Dice similarity coefficient (F, proportion of share fragments between two isolates). F value of 1.0 indicates identical pattern, and F value of 0 suggests complete dissimilarity.[11,12] Electrophoretic patterns were analysed for relatedness using a computer program Numerical Taxonomy and Multivariate Analysis (NTSYS-pc) version 1.80.[13] Dendograms were constructed by using Dice coefficient, and clustering by using unweighted pair group arithmetic means method (UPGMA).

RESULTS

A total of 25 human isolates of *S.typhi* were analysed following digestion with *Xba*I (5'-TCTAGA-3') restriction endonuclease and electrophoresis using PFGE. Digested chromosomal DNAs produced 10 to 20 fragments with molecular weight ranging from 30 kb to 650 kb and showed various PFGE patterns as shown in Fig. 1A and 1B. Close examination to these bands showed that DNA fragments with molecular weight between 31 kb to 97 kb, and around 340 kb were relatively conserved in most of the isolates. However, bands between 97 kb to 340 kb, and above 340 kb were more various. Analysis of genetic relatedness among *S.typhi* isolates showed a quite significant diversity among them as represented by their F values (Fig. 2A), and the presence of 18 different PFGE types (pulsotypes) designated X1 to X18, and 4 subtypes as follows: X1.1, X1.2, X2.1 and X2.2 (see Table 1). Despite the divergence of F values, certain pulsotypes were either identical or very similar, in that they were different in only 1 or 2 bands. There were 5 *S.typhi* isolates (isolate no. 3, 23, 43, 44 and 52) with F value between 0.923–1.000, and therefore they were grouped together in PFGE cluster II. Three of these isolates, i.e. isolate no. 23, 43 and 52 were identical with F value 1.000. Moreover, 4 other isolates (isolate no. 46, 48, 55 and 56) showed high degree of similarity with F between 0.941–0.971, and were grouped in cluster I (see Fig 2A and 2B).

(A)



(B)

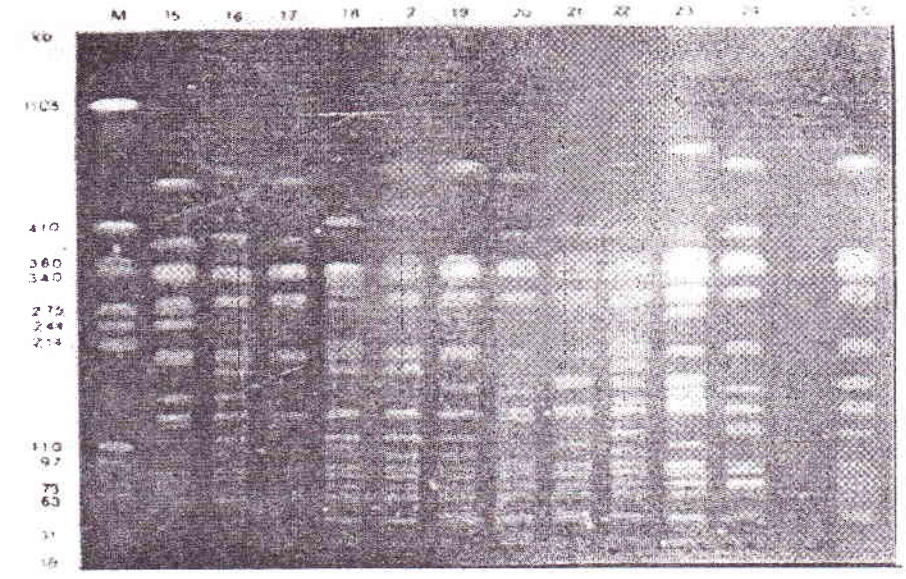


Figure 1. Agarose gel showing XbaI digested DNA of S. typhi isolates originated from hospitalised, sporadic typhoid fever patients. (A) Lanes 1-14 represent restriction enzyme digested DNA patterns of isolate number: 55, 29, 39, 23, 49, 18, 45, 43, 46, 32, 44, 33, 52, and 47. (B) Lanes 15-25 represent isolate number: 63, 54, 50, 51, 45, 56, 58, 2, 3, 53, 59, and 48. Lane M: PFGE marker (Rhodobacter sphaeroides 2.4.1. digested with AseI) in kb pairs.

(A).

55 1.000
29 0.514 1.000
39 0.667 0.703 1.000
23 0.914 0.500 0.757 1.000
49 0.629 0.722 0.865 0.667 1.000
18 0.765 0.571 0.778 0.800 0.686 1.000
45 0.882 0.514 0.722 0.914 0.686 0.765 1.000
43 0.914 0.500 0.757 **1.000** 0.667 0.800 0.914 1.000
46 0.971 0.556 0.703 0.889 0.667 0.800 0.857 0.889 1.000
32 0.722 0.649 0.895 0.757 0.757 0.833 0.667 0.757 0.757 1.000
44 0.889 0.486 0.737 0.973 0.649 0.833 0.944 0.973 0.865 0.737 1.000
33 0.757 0.526 0.821 0.842 0.789 0.865 0.757 0.842 0.789 0.821 0.821 1.000
52 0.914 0.500 0.757 **1.000** 0.667 0.800 0.914 **1.000** 0.889 0.757 0.973 0.842 1.000
47 0.722 0.649 0.895 0.757 0.811 0.778 0.833 0.757 0.757 0.789 0.789 0.769 0.757 1.000
63 0.686 0.778 0.757 0.667 0.722 0.743 0.629 0.667 0.722 0.757 0.649 0.684 0.667 0.649 1.000
54 0.788 0.529 0.800 0.882 0.706 0.727 0.848 0.882 0.765 0.743 0.857 0.778 0.882 0.743 0.706 1.000
50 0.667 0.765 0.743 0.647 0.588 0.727 0.606 0.647 0.706 0.743 0.629 0.667 0.647 0.686 0.882 0.625 1.000
51 0.667 0.588 0.857 0.706 0.882 0.727 0.727 0.706 0.706 0.743 0.686 0.833 0.706 0.857 0.647 0.688 0.625 1.000
56 0.941 0.571 0.667 0.857 0.686 0.765 0.824 0.857 0.971 0.722 0.833 0.757 0.857 0.722 0.686 0.727 0.667 0.667 1.000
58 0.606 0.824 0.800 0.647 0.706 0.727 0.606 0.647 0.647 0.800 0.629 0.667 0.647 0.686 0.882 0.688 0.875 0.625 0.606 1.000
2 0.800 0.556 0.703 0.778 0.611 0.857 0.686 0.778 0.833 0.757 0.757 0.842 0.778 0.649 0.778 0.706 0.765 0.647 0.800 0.706 1.000
3 0.865 0.526 0.769 0.947 0.684 0.811 0.865 0.947 0.895 0.769 0.923 0.850 0.947 0.769 0.684 0.833 0.667 0.722 0.865 0.667 0.789 1.000
53 0.684 0.615 0.650 0.718 0.615 0.789 0.632 0.718 0.718 0.700 0.700 0.780 0.718 0.600 0.718 0.649 0.649 0.595 0.684 0.703 0.872 0.780 1.000
59 0.765 0.629 0.889 0.800 0.743 0.765 0.765 0.800 0.743 0.889 0.778 0.757 0.800 0.833 0.743 0.848 0.727 0.727 0.706 0.788 0.686 0.757 0.632 1.000
48 0.941 0.571 0.722 0.914 0.686 0.824 0.882 0.914 0.971 0.778 0.889 0.811 0.914 0.778 0.686 0.788 0.667 0.727 0.941 0.667 0.800 0.919 0.737 0.765 1.000

Figure 2. (A). Matrix of F values of 25 S. typhi isolates. The far left ordinate represents the isolate's number.

(B).

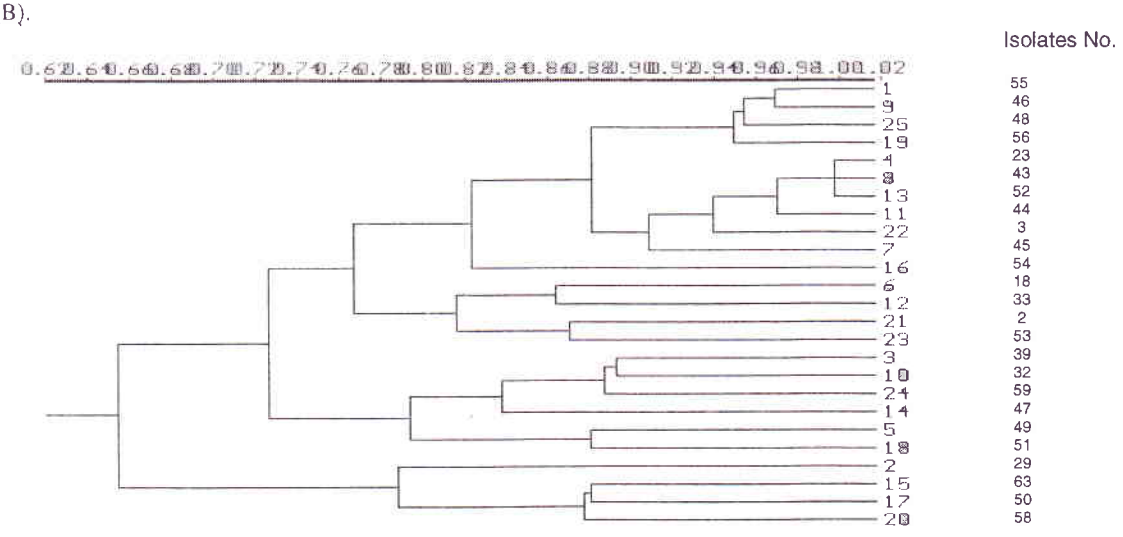


Figure 2. (B) Dendogram showing the clusters derived from the 25 S. typhi isolates, which was constructed by the NTSYS-pc version 1.8 program on the basis of F values and the unweighted pair group arithmetic means methods (UPGMA). Scale indicates level of genetic relatedness between collection of isolates.

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