Original Article

The molecular epidemiology of *Salmonella* Typhi across Indonesia reveals bacterial migration

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Abstract

Background: Typhoid fever remains a worldwide problem, but it is particularly common in Southeast Asia, including Indonesia. The causative agent *Salmonella* Typhi is known to have significant genome plasticity.

Methodology: This study describes genetic fingerprints using restriction fragment length polymorphism with *Spe*I of chromosomal DNA and pulsed-field gel electrophoresis (PFGE) of *S.* Typhi isolated from different geographic areas spreading from west to east across Indonesia.

Results: A total of 33 *Spe*I digested *S.* Typhi chromosomal DNA gave 22 schizotypes, 20 pulsotypes, and 12 subtypes indicating genomic diversity and the presence of more than one clone of *S.* Typhi. Cluster analysis at a degree of similarity of ≥ 80% showed four clusters, three of which were associated with geographic area. One cluster (Dice coefficient 0.727-1.000) contained isolates from three different geographic areas (Jakarta, Makasar, Jayapura), spread across Indonesia.

Conclusions: Genetic fingerprinting of *S.* Typhi in Indonesia showed the presence of endemic strains in localized geographic areas, as well as the movement of one strain type throughout the archipelago.

Key words: *Salmonella* Typhi, Typhoid fever, pulsed-field gel electrophoresis, molecular epidemiology

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Introduction

Typhoid fever is a major health problem in Southeast Asian countries including Malaysia, Thailand, and Indonesia [1]. In 2007, the Communicable Disease Centre (CDC) of Indonesia reported a prevalence of 358-810 per 100,000 population for typhoid fever with 64% occurring in 3- to 19-year olds [2]. In South Sulawesi, the case detection rate increased from 257 per 100,000 population in 1991 to 386 per 100,000 population in 2007 [3]. In Jakarta, typhoid fever was the second leading infectious disease after gastroenteritis and caused the highest mortality [4]. The mortality rate varied from 3.1-10.4% among hospitalized patients [2] with cases occurring throughout the year but peaking in the dry season. In rural areas there are probably many cases that remain undiagnosed due to limited diagnostic facilities. Studies of the molecular epidemiology of *S.* Typhi have been conducted since the early nineties using pulsed-field gel electrophoresis (PFGE) and have shown significant genetic diversity of *S.* Typhi isolates both from outbreaks and sporadic cases of typhoid fever [5,6,7,8,9]. An increase in the number of people who travel domestically and internationally also provides a means by which *S.* Typhi can migrate from one place to another, and may contribute to the emergence of new strains of *S.* Typhi. Previous investigations showed the existence of multiple strains of *S.* Typhi in Jakarta which simultaneously co-existed causing endemic and sporadic disease [7,10,11]. In this study we used PFGE to analyze the genetic diversity of *S.* Typhi isolates originating from patients with typhoid fever from five different geographic areas across Indonesia.

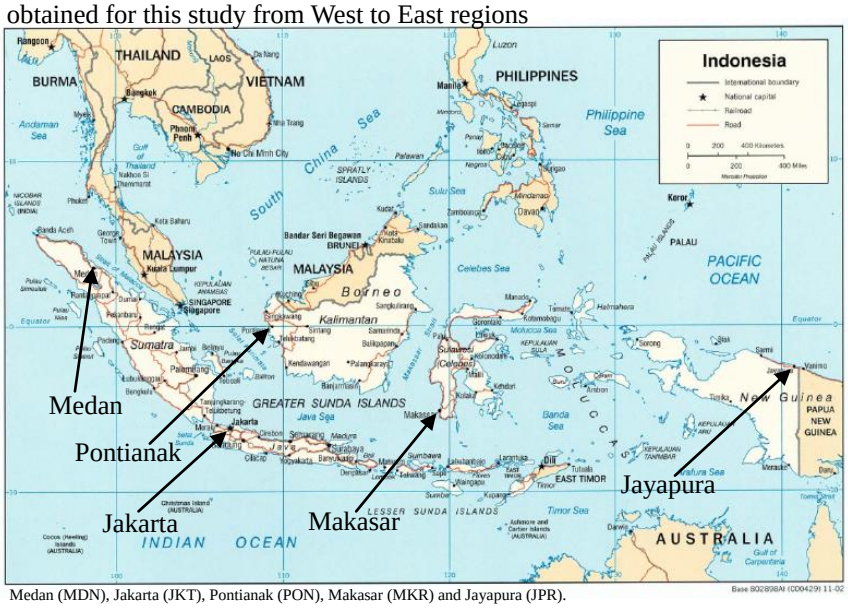
Methods

The investigation was conducted in the laboratory of the Department of Microbiology in the Faculty of Medicine at the University of Indonesia in Jakarta.

Bacterial isolates

S. Typhi isolates were taken from collections stored in the Department of Microbiology, Faculty of Medicine, at the University of Indonesia, and from the Center of Health Research and Development, Ministry of Health, Republic of Indonesia. Out of a total of 33 *S.* Typhi isolates, 10 isolates originated from sporadic typhoid fever cases in Jakarta (JKT), 9 from Pontianak (PON), 3 from Makasar (MKR), 10 from Jayapura (JPR) and one from Medan (MDN). All isolates were retested using standard biochemical reactions, and commercial slide agglutination from Biofarma Indonesia. Susceptibility of the bacteria to

Figure 1. Map of Indonesia showing the locations from which *S.* Typhi was obtained for this study from West to East regions



Medan (MDN), Jakarta (JKT), Pontianak (PON), Makasar (MKR) and Jayapura (JPR).

antibiotics was examined using the disc diffusion method. The five cities from which the isolates were obtained are located in different islands. Medan, Jakarta and Pontianak are located in Sumatra, Java and Kalimantan Islands respectively, which are all in West Indonesia. Makasar is located in Sulawesi Island, which is in Central Indonesia, and Jayapura is in Papua, which is in East Indonesia (see Figure 1).

Pulsed-Field Gel Electrophoresis (PFGE) and Genome Analysis

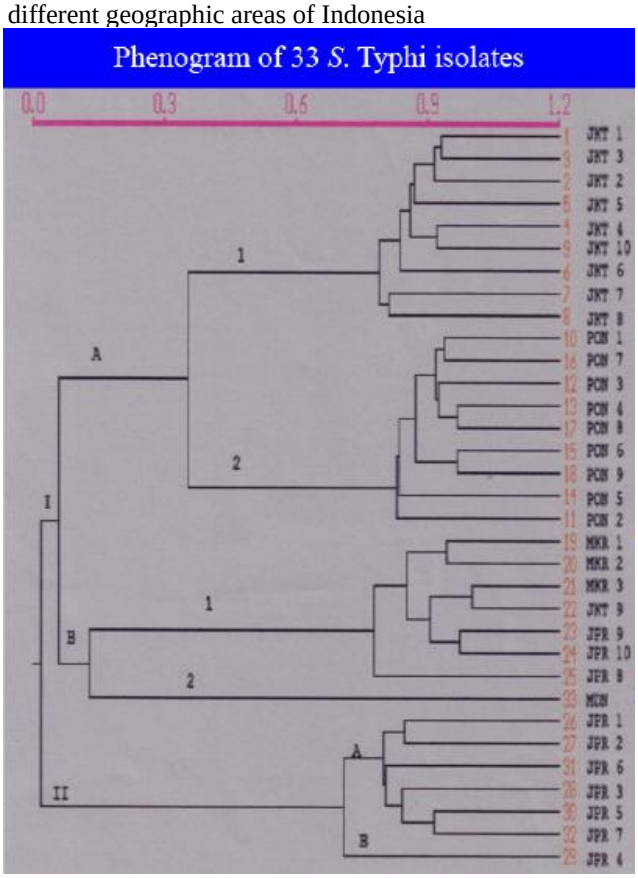
Preparation of the DNA genome was conducted as described earlier by Suwanto and Kaplan [12] and Thong *et al*. [5]. Pulsed-field gel electrophoresis (PFGE) of *Spe*I (5' ACTAGT-3') digested chromosomal DNA was performed as in Moehario and Soemanto [10], in which PFGE CHEF-DR II (Bio-Rad Laboratories, Richmond, California, USA), program 17(50-900) for *Salmonella sp*. was used. Electrophoresis was performed at 14°C for approximately 19.5 hours with pulse time 5.3 to 60 seconds and electrical current of 6 volt per cm^2. DNA restriction patterns resulting from rare-cutting endonuclease were designated as schizotypes (Suwanto and Kaplan) [13]. Interpretation of the PFGE patterns was performed as described earlier by Bannerman *et al*.[14] and assigned for arbitrary pattern types and compared by calculating Dice similarity coefficient. PFGE type (pulsotype) and subtype were based on criteria by Bannerman *et al*. [14] and Zadoks *et al*., [15]. *S.* Typhi isolates showing the same PFGE patterns or differing by one to three bands were grouped in the same types while those with PFGE patterns differing up to four to six bands were grouped as closely related types. Different or unrelated types were determined if PFGE patterns differed by seven bands or more. The designation of subtypes was used for those which had up to three PFGE band differences. A cluster analysis was performed using unweighted pair group arithmetic means method (UPGMA) of Dice coefficient for all pairs of strains, and a phenogram was generated using the computer program Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version 1.80 (Rohlf) [16].

Results

A total of 33 chromosomal DNA extractions from *S.* Typhi digested with *Spe*I restriction endonuclease were analyzed after electrophoresis using a CHEF-DR II machine (BioRad, USA). The DNA profiles of these isolates showed PFGE patterns with distinctive differences according to their regions, *i.e.*, Jakarta, Pontianak, Makasar, Jayapura and Medan. The electrophoresis fragments were unique for each of the isolates and therefore they are the fingerprints for each of them. Nonetheless, all the *S.* Typhi isolates showed similar phenotypes in that they were susceptible to three antibiotics tested, *i.e.*, Chloramphenicol, Ampicilin and Cotrimoxazole. The digestion of the DNA genome with *Spe*I resulted in 12 to 23 DNA fragments with molecular weight ranges between 29 and 1,100 kb. Estimated genome size varied from 1,495 up to 4,516 kb.

Genetic similarity and dissimilarity of *S.* Typhi were examined. Ten *S.* Typhi isolates from Jakarta showed close genetic relatedness except for one isolate. Cluster analysis showed that nine isolates (JKT1, JKT3, JKT2, JKT5, JKT4, JKT10, JKT6, JKT7 and JKT8) were closely related and grouped together in group IA1 (see Figure 2). Dice coefficients of these isolates ranged from 0.737 to

Figure 2. Phenogram of 33 *S*. Typhi isolates from five different geographic areas of Indonesia



Jakarta (JKT), Pontianak (PON), Makasar (MKR), Medan (MDN), Jayapura (JPR)

0.930. JKT9 had a different PFGE profile from the other nine isolates, with Dice coefficients 0.143-0.205. At the ≥ 80 % degree of similarity, all 10 JKT isolates gave F value ranging from 0.143-0.930. The cluster analysis demonstrated that JKT9 did not share genetic relatedness with the other nine isolates, and therefore it is unlikely that it originated from Jakarta. Nine *S.* Typhi isolates from Pontianak all gave closely related PFGE patterns, and they were all clustered in IA2 (Figure2). All nine isolates had Dice coefficients ranging from 0.765-0.968 thus suggesting these isolates were homogenous. Three *S.* Typhi isolates from Makasar were also very closely related with Dice coefficients 0.857-0.941. All the Makasar isolates (MKR1, MKR2, and MKR3) were grouped in cluster IB1 together with isolates derived from other areas as follows: JKT9 from Jakarta; JPR9, JPR10, and JPR8 from Jayapura (Figure 2). These seven isolates seemed to be closely genetically related (F value 0.727-0.971). One isolate from Medan (MDN) was included in cluster IB2. It was dissimilar to those in cluster IB1, and the Dice coefficient was 0.125-0.138. Six of the *S.* Typhi isolates from Jayapura (JPR1, JPR2, JPR6, JPR3, JPR5, and JPR7) were clustered together in cluster IIA and seemed to be closely related with Dice coefficients 0.774-0.941. JPR4, however, showed an F value of 0.621-0774 to those six isolates, and thus was grouped by itself in IIB. At the ≥ 80% degree of similarity, all 10 isolates from Jayapura had diverse genome profiles, with F values ranging from 0.000-0.971. Three isolates of Jayapura (JPR 8, JPR9, and JPR10) apparently were not related to those in cluster IIA and IIB, which is shown by their Dice coefficients (0.000-0.069). The genetic fingerprints for ten *S.* Typhi isolates of Jayapura showed that although the bacteria had originated from the same area, they might not have derived from the same ancestor. The Dice coefficient for all *S. Typhi* isolates from five different geographic areas across Indonesia ranged from 0.000-0,971 thus indicating the heterogeneity of *S.* Typhi in the country.

Of all 33 isolates of *S.* Typhi, 22 schizotypes, 20 pulsotypes and 12 subtypes were found (see Table 1). *S.* Typhi isolates included in cluster I A1 exhibited six schizotypes, six pulsotypes, and three subtypes, while those in cluster I A2 had six schizotypes, four pulsotypes, and five subtypes. Cluster I B1, consisting of collections of schizotypes from *S.* Typhi isolates from three areas (Makasar, Jakarta and Jayapura) revealed four schizotypes, four pulsotypes, and two subtypes. Interestingly, two *S.* Typhi isolates originating from two different areas, Makasar and Jakarta, were identical (Table 1). These two isolates, JKT9 and MKR3, might have originated from one clone. Cluster II A exhibited four schizotypes, four pulsotypes, two subtypes and one schizotype, while cluster II B exhibited one pulsotype.

Discussion

A total of 33 isolates of *S.* Typhi were examined. The isolates originated from five locations in three regions of Indonesia: the west region (Medan, Jakarta, Pontianak); the central region (Makasar); and the east region (Jayapura). All 33 isolates were examined for their genetic relatedness using PFGE. Numbers of DNA fragments obtained in this study were similar to those of our previous study of genomic digestion by *Xba*I [10,11]. DNA fragments with molecular weight 97 kb and lower remained consistent, while DNA fragments between 97-360 kb and above 360 kb were varied [10,11]. Genome size of these isolates was varied from 1,495 up to 4,516 kb. The smallest genome size was found in the *S.* Typhi isolate originating from Jayapura (JPR) and the largest was the isolate from Jakarta (JKT). Thong *et al*. used three different restriction enzymes, *Xba*I, *Avr*II, and *Spe*I, and found that the genome sizes of *S.* Typhi differed by as much as 959 kb, ranging from 3,964 to 4,923 kb (mean genome size 4,528 kb) [17]. Pang [9] reported the analysis of *S.* Typhi genomes derived

Table 1. Schizotypes and Pulsotypes of *Salmonella* Typhi genome digested with SpeI

Clusters	Isolates	Schizotypes	Pulsotypes	Subtypes
I A1	JKT 1	1	1	
	JKT 3	1		1.1
	JKT 2	2		1.2
	JKT 5	3	2	
	JKT 4	4	3	
	JKT 10	4		3.1
	JKT 6	5	4	
	JKT 7	6	5	
	JKT 8	6	6	
I A2	PON 1	7	7	
	PON 7	7		7.1
	PON 3	8		7.2
	PON 4	9		7.3
	PON 8	9		7.4
	PON 6	10	8	
	PON 9	10		8.1
	PON 5	11	9	
	PON 2	12	10	
I B1	MKR 1	13	11	
	MKR 2	13		11.1
	MKR 3	14	12	identical
	JKT 9	14	12	identical
	JPR 9	15	13	
	JPR 10	15		13.1
	JPR 8	16	14	
I B2	MDN	17	15	
II A	JPR 1	18	16	
	JPR 2	18		16.1
	JPR 6	19	17	
	JPR 3	20	18	
	JPR 5	21	19	
	JPR 7	21		19.1
II B	JPR 4	22	20	

from many countries including Indonesia, Papua New Guinea, Chile, and Malaysia. The genome size of S. Typhi from Indonesia was 4,750-4,860 kb, from Papua New Guinea it was 3,980-4,180 kb, from Chile it was 4,330-4,660 kb, and from Malaysia it was 4,620-4,870 kb. Differences in genome size were probably due to genetic events such as chromosome rearrangements and mutations; nonetheless, area of origin might also be considered. The S. Typhi genome has been shown to rearrange more frequently compared to other enteric bacteria during evolution [18,19]. PFGE has been used in many studies to show the presence of genome diversity and confirm genomic plasticity of S. Typhi [5,7,9,10,11,17,20]. This study demonstrated the genomic heterogeneity of S. Typhi isolated from many areas in Indonesia. The study also showed that the isolates were not derived by clonal expansion from a single source. Despite being heterogeneous genetically, all 33 S. Typhi isolates showed distinguishable phenotypes and were susceptible to all three antibiotics tested.

An interesting observation was that genome analysis using PFGE could detect mobility and movement of *S.* Typhi. Those isolates included in cluster IB1 possibly originated from East Indonesia, except JKT9, which might have occurred in Jakarta due to the migration of people from east to west Indonesia. A previous investigation reported that *S.* Typhi isolates from Indonesia were found to share some characteristics with those from Malaysia and Thailand [5]. Similarity and dissimilarity of *S.* Typhi isolates from Indonesia, Malaysia and Thailand showed that not only was the *S.* Typhi genome plastic, but also that there was a migration of *S.* Typhi in Southeast Asia [6, 7]. Additionally, PFGE techniques were able to detect particular *S.* Typhi strains which travelled quite a distance to El Salvador, Mexico, and Bangladesh [21].

In conclusion, DNA fingerprints of *S.* Typhi produced by PFGE showed the presence of endemic strains in localized geographic area in Indonesia as well as the movement of one strain type throughout the archipelago.

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Conflict of interest: No conflict of interest is declared.