CLONAL RECONQUEST OF ANTIBIOTIC-SUSCEPTIBLE SALMONELLA ENTERICA SEROTYPE TYPHI IN SON LA PROVINCE, VIETNAM

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Abstract. In the last three decades, high rates of resistance to common first-line antimicrobial agents have been reported in Salmonella enterica serotype Typhi (Typhi), the causative organism of typhoid fever (TF), in many regions of the world, especially in South East Asia. Analysis of Typhi strains isolated from outbreaks and sporadic cases of TF in Son La province, northwest Vietnam, in 2002 revealed that 94.5% (85/90) of the isolates were fully susceptible to amoxicillin, chloramphenicol, cotrimoxazole, tetracycline, and nalidixic acid. There was a clear decline in the occurrence of multi-drug resistant (MDR) Typhi isolates collected in this province in 2002 (4.4%) compared with the period 1995–1999 in the same province (30.8–100%). By using molecular (IS2000 profiling, PsiI-ribotyping, XbaI-pulsed-field gel electrophoresis, and haplotyping) and phage-typing methods, we showed that the Typhi isolates from Son La province in 2002 were genetically related; however, they were unrelated to the previous MDR clones established in Vietnam.

INTRODUCTION

Typhoid fever (TF) remains a major health problem in the world, with an estimate of > 20 million cases resulting in > 200,000 deaths during 2000, mostly in developing countries. Treatment with appropriate antibiotics is essential for recovery. However, treatment has become progressively more problematic with the gradual emergence of antimicrobial resistance. In the last three decades, high rates of resistance to common first-line antimicrobial agents have been reported in Salmonella enterica serotype Typhi (hereafter referred to as Typhi) in many regions of the world. Multiple resistance to ampicillin, chloramphenicol, cotrimoxazole, and tetracycline (ACStXTe resistance type) is encoded by large conjugative plasmids mostly belonging to the incompatibility complex group IncHI.3–6 The Indian subcontinent and Southeast Asian countries are particularly affected by multidrug resistant (MDR) Typhi strains. In Vietnam, the spread of MDR Typhi (ACStXTe R-type) was first reported in 1992–1993 in the southern part of the country. In 1994, > 80% of Typhi isolates were MDR in southern Vietnam, but only 5% and 10% of MDRST strains were isolated in the center and the north, respectively. During the period 1995–2002, a study found that > 90% of sporadic and epidemic Typhi strains from the center and the north were MDR. The economic reforms in Vietnam in the early 1990s had resulted in a boom in private pharmacies and all first line antibiotics for TF could be bought as over-the-counter medicines without prescription, leading to misuse and abuse of these drugs. Because of the widespread occurrence of MDR Typhi strains, quinolones and fluoroquinolones, in particular, were used in the first-line treatment of adults in several countries, including Vietnam. However, the emergence of MDR Typhi isolates with an additional chromosomally encoded resistance to nalidixic acid (MDR-NalR) and with reduced susceptibility to ciprofloxacin has been increasingly reported since the beginning of the 1990s on the Indian subcontinent and afterwards in different Asian countries. Several reports indicated that MDR-NalR Typhi strains were associated with slower clinical responses to fluoroquinolones or treatment failures. In Vietnam, MDR-NalR Typhi isolates were first reported in 1993 and increased dramatically in 1997 in the south of the country. As outbreaks of TF are reported every year in various parts of Vietnam, the emergence of MDR-NalR Typhi isolates is of great concern because TF caused by such isolates would require treatment with expensive, third-generation cephalosporins that are unaffordable for most people in Vietnam. Furthermore, the average cost of admission to Vietnamese hospitals (including bed fees, health care professionals, and cost of treatment) for a patient infected with MDR-NalR Typhi has been estimated to be USD 50 compared with USD 22 for a patient infected with a susceptible strain.

In northern Vietnam, MDR Typhi strains were reported in most provinces: Lao Cai, Lai Chau, Thanh Hoa, and Son La, since the mid-1990s (H. Tran, unpublished results). From July to December 2002, a hospital-based study aiming at identifying risk factors associated with TF in Son La province was conducted by some of us (H. Tran, unpublished results). From July to December 2002, a hospital-based study aiming at identifying risk factors associated with TF in Son La province was conducted by some of us (H. Tran, unpublished results). From July to December 2002, a hospital-based study aiming at identifying risk factors associated with TF in Son La province was conducted by some of us (H. Tran, unpublished results). From July to December 2002, a hospital-based study aiming at identifying risk factors associated with TF in Son La province was conducted by some of us (H. Tran, unpublished results). From July to December 2002, a hospital-based study aiming at identifying risk factors associated with TF in Son La province was conducted by some of us (H. Tran, unpublished results).
comparative study (antibiotyping, molecular, and phage typing) of S. enterica serotype Typhi isolates collected during the previous study to explore how this could help to understand the mode of acquisition of TF in Son La province in 2002.

MATERIALS AND METHODS

Study place. The research was conducted in Son La province, in northwest Vietnam, 320 km from the capital Hanoi. This province, 80% covered by mountains, is difficult to access. Son La town is the main town of this province, subdivided into nine districts. The standard of living of the population is very low, and income per capita is among the poorest compared with other provinces in Vietnam (USD 130/person/year).23 TF is of great concern in Son La province with outbreaks reported in several districts from 1998 to 2001.

Patients and sampling. The patients in this study were recruited for a confirmed TF in provincial and district hospitals of Son La province between 1 July and 30 December 2002, as reported previously.22 One blood and one stool sample were obtained for each patient. Diagnosis of TF was made by isolation of Typhi from blood and/or from stool associated with clinical symptoms compatible with recent TF infection, i.e. fever over 38°C for more than 3 days with no other evident diagnosis to explain this fever.

Origin and identification of Typhi isolates. Blood samples (5 mL for patients older than 5 years old, and at least 2 mL for children under 5 years old) were inoculated into Brain Heart Infusion broth (Difco, Detroit, MI) and incubated at 35–37°C for 10 days. Vials were checked for growth twice daily on the first 2 days, once on day 3 and day 4, and once on day 10. Positive vials were subcultured on blood, MacConkey, and Salmonella-Shigella agar plates (SS agar, Difco). A stool sample was collected at the same time as the blood sample. Stool (3 g) was inoculated into selenite broth enrichment medium (Difco), incubated at 35–37°C for 18–24 hours and then subcultured on MacConkey and SS agar. Identification of Typhi was performed in Son La Health Center Microbiology Department using biochemical tests and agglutination with O, H, and Vi antisera (Difco). Suspected Typhi strains were sent for confirmation to the National Reference Laboratory of Enteric Pathogens, National Institute of Hygiene and Epidemiology (NIHE), Hanoi, Vietnam. Molecular and phage typing studies were carried out at the French National Reference Center for Salmonella (NRC-Salm), Institut Pasteur, Paris, France. Typhi strains 162/95, 230/95, 14/96, 339/95, 358/98, and CM2664 collected in Vietnam from 1995 to 2002 and used as comparison strains (CS) were from the NRC-Salm collection. Typhi reference strain Ty-2 was from the WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur, Paris, France.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on Typhi isolates from Son-La, 2002 using the Paper Disc Method (PDM-Biodisk, Stockholm, Sweden) at the National Reference Laboratory for Enteropathogenic Bacteria, Norwegian Institute of Public Health, Oslo, Norway, using Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) guidelines.24,25 The following disks were used: amoxicillin (10 μg), amoxicillin + clavulanic acid (20 μg + 10 μg), streptomycin (30 μg), tetracycline (30 μg), ceftriaxone (30 μg), azithromycin (15 μg), chloramphenicol (30 μg), sulfamethoxazole (23.8 μg), trimethoprim (5 μg), nalidixic acid (30 μg), norfloxacin (10 μg), and ciprofloxacin (10 μg). Escherichia coli ATCC 25922 was used as a control.

Pheage typing. Vi-phage typing of the 90 Typhi isolates from Son La province, 2002 and of the eight CS followed a standardized methodology as described previously.26 Phage suspensions were kindly provided by the Health Protection Agency (Colindale, United Kingdom).

IS2000 profiling. IS2000 profiling using PstI (Roche, Mannheim, Germany) for the cleavage of the genomic DNA was performed on 33 selected Typhi isolates from Son La province, 2002, and on eight CS, as described previously.26

Ribotyping. The membranes used for IS2000 profiling were reprobed with digoxigenin (DIG)-labeled OligoMix5 probe as described previously.20 Fifteen Typhi isolates, which exhibited different representative ribotypes, were subjected to the RiboPrinter microbial characterization system (Qualicon, Wilmington, DE), a fully automated and standardized ribotyping method for creating a database. Ribotype numbering was generated by this system. Image normalization and construction of similarity matrices were carried out using BioNumerics 4.0 (Applied Maths, Sint-Martens-Latem, Belgium). Ribotype profiles were compared with the RiboPrinter database of the NRC-Salm (1997–2004, 339 PstI-ribotypes of Typhi).

Pulsed-field gel electrophoresis. Pulsed-field gel electrophoresis (PFGE) of XbaI (Roche)-digested genomic DNA was carried out on a subset of 29 Typhi from Son La, 2002, and on eight CS, as described previously.22 The running conditions and the molecular size marker (XbaI-digested DNA from S. enterica serotype Braenderup H9812) were the same as described in the standardized PulseNet protocol.28 Image normalization and construction of similarity matrices were carried out using BioNumerics 4.0. Bands were assigned manually, and clustering was performed using the unweighted pair-group method with arithmetic averages (UPGMA) based on the Dice similarity index, utilizing an optimization parameter of 0.5% and a 1% band-position tolerance. Each profile that differed by one or more bands was assigned a type.

Haplotyping by denaturing high-performance liquid chromatography (DHPLC) analysis. Fifty-two polymorphic coding gene fragments (Table 1) were amplified from 37 strains, including a subset of 31 Typhi from Son La and 6 CS, as described previously.29 PCR products were amplified over 25 cycles in 25 μL volumes, containing 15 ng of DNA from each of 4 test strains plus a reference strain (CT18), polymerase (1.25 units, Optimase, Transgenomic, Omaha, NE), as well as specific primers (320 nM, Table 1) and dNTPs (0.2 mM). Duplex obtained after the annealing of denatured PCR fragments were analyzed by DHPLC with a DNA-SepR Cartridge (WaveR Nucleic Acid Fragment Analysis System, Transgenomic) at the temperatures indicated in Table 1. Ac-
According to the DHPLC profiles, representative PCR products showing evidence of mutations were purified and sequenced from both strands by Agowa (Berlin, Germany).

RESULTS

Origin and antimicrobial susceptibility of Typhi isolates. A total of 90 Typhi isolates (49 from blood culture only, 8 from both blood and stool culture, and 33 from stool culture only) were recovered from 90 patients with confirmed TF among the 617 patients who were admitted with a suspected TF in Son La province hospitals during the time of the study. None of the patients infected with Typhi reported any travel outside the province during the incubation period. The isolates were recovered from patients living in four different areas: Thuan Chau district (N/H11505 28), Phu Yen district (N/H11505 32), Quynh Nhai district (N/H11505 23), and Son La town (N/H11505 7).

Molecular epidemiology of Typhi isolates. The DHPLC profiles of Typhi isolates were used to identify polymorphic gene fragments. Table 1 shows the polymorphic gene fragments tested by denaturing HPLC analysis on Typhi strains isolated from Son La province, Vietnam, 1995–2002.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fragment length (bp)</th>
<th>DHPLC temp (°C)</th>
<th>5′-3′ Forward/reverse primers</th>
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<tr>
<td>STY0006</td>
<td>483</td>
<td>60.7</td>
<td>CAGTTTCCCCGTTGGCACGCT/TCGTATCCCCCTCCACCTCCT</td>
</tr>
<tr>
<td>STY0135</td>
<td>497</td>
<td>60.8</td>
<td>TACGTTAGCTGACAGACCG/AGCTAAGAGATGGGAGCCGTCG</td>
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<tr>
<td>STY0175</td>
<td>428</td>
<td>61.4</td>
<td>CATATGCTTCCGGCGGATCTA/CAATGCTGCAGGTCG</td>
</tr>
<tr>
<td>STY0214</td>
<td>482</td>
<td>63.2</td>
<td>GGACGACGTCATGATGGGCG/CCGGCTAGCAAGCTCCAGCAT</td>
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<tr>
<td>STY0321</td>
<td>440</td>
<td>64.0</td>
<td>GTTACGCTACCGGCGAAGAG/AGCTAAGAGATGGGAGCCGTCG</td>
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<tr>
<td>STY0336</td>
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<td>CACCCGAGCAGTACGTTT/AGAGCTAAGAGATGGGAGCCGTCG</td>
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<td>STY0573</td>
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<td>62.9</td>
<td>GATACGCTACGCGGAG/GGTAAGGTAGAGATGGGAGCCGTCG</td>
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<tr>
<td>STY0831</td>
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<td>62.9</td>
<td>CCCGCGACATGACGCTGAC/CGTATCGACGCTGACGCTGACGCTG</td>
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<tr>
<td>STY1061</td>
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<td>61.4</td>
<td>CTCGTAAGTCCGGCGCTACG/AGCTAAGAGATGGGAGCCGTCG</td>
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<td>STY1121</td>
<td>500</td>
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<td>STY1121</td>
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<td>61.5</td>
<td>ACAAGCAGGACAGTCTCAG/AGCTAAGAGATGGGAGCCGTCG</td>
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<tr>
<td>STY2575</td>
<td>438</td>
<td>61.7</td>
<td>GCAGGACAGAGGACAGTCTCAG/AGCTAAGAGATGGGAGCCGTCG</td>
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</tbody>
</table>

Table 1
Typhi isolates collected in this province in 2002 were fully susceptible to amoxicillin, chloramphenicol, trimethoprim, tetracycline, and nalidixic acid (Table 2). Four isolates (4.4%) displayed single resistance to amoxicillin. One isolate displayed single resistance to sulfamethoxazole (1.1%). When compared with the data of 116 Typhi strains collected during routine surveillance in Son La province from 1995 to 1999, the results clearly indicate a decline in the occurrence of MDR compared with the data of 116 Typhi strains collected during weeks 36–40 (September–October).

Antimicrobial susceptibility testing revealed that 94.5% (85/90) of the Typhi isolates collected in 2002 were fully susceptible to amoxicillin, chloramphenicol, trimethoprim, tetracycline, and nalidixic acid (Table 2). Four isolates (4.4%) displayed single resistance to amoxicillin. One isolate displayed single resistance to sulfamethoxazole (1.1%). When compared with the data of 116 Typhi strains collected during routine surveillance in Son La province from 1995 to 1999, the results clearly indicate a decline in the occurrence of MDR compared with the data of 116 Typhi strains collected during weeks 36–40 (September–October).

Molecular and phage typing of Typhi isolates. Vi-phage typing was done for the 90 Typhi isolates from Son La prov-ince, 2002. All but five (94.4%) were of phage type A. Four isolates were degraded Vi-strain (DVS) and one was Vi-negative (Table 2).

For the molecular typing study, 33 Typhi isolates from Son La province were selected: 9 from Phu Yen (N = 32), 10 from Thuan Chau (N = 28), 7 from Quynh Nhai (N = 23), and 7 from Son La town (N = 7). We used four molecular methods to study the genotypic relationship among these isolates: Pst-IS200 typing, Pst-ribotyping, XhoI-PFGE (only 29 isolates were typed by PFGE), and the newly described haplotyping by DHPLC.

To compare the Typhi genotypes circulating in Son La province in 2002 to other genotypes previously observed in northern provinces or currently observed in southern Vietnam, we have also tested seven CS recovered from 1995 to 2002 in different provinces of Vietnam and displaying various antimicrobial-resistance phenotypes (Table 3).

Only two Pst-IS200 profiles were observed in our study: profile IS1 was present in the 33 selected Typhi isolates from Son La province, whereas profile IS2 was observed in 8 CS (including reference strain Ty-2) (Table 3, Figure 1A).

Fifteen Pst-ribotypes were found: 10 (S007, 177, 340, 343-349) in Son La province isolates and 5 (03a, 26a, 187, 236 and 73/34/8) in CS (Table 3, Figure 1B). Ribotype 344 was the most frequently observed ribotype in Son La province isolates (19/33, 57.6%), whereas it was not found in CS.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>1995 (n = 15)</th>
<th>1996 (n = 27)</th>
<th>1997 (n = 28)</th>
<th>1998 (n = 33)</th>
<th>1999 (n = 15)</th>
<th>2002 (n = 50)</th>
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<tr>
<td>Amoxicillin</td>
<td>100</td>
<td>63.0</td>
<td>92.9</td>
<td>72.7</td>
<td>30.8</td>
<td>4.4</td>
</tr>
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<td>Ceftriaxone</td>
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<td>0</td>
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<tr>
<td>Tetracycline</td>
<td>100</td>
<td>66.7</td>
<td>92.9</td>
<td>63.6</td>
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<td>Cotrimoxazole</td>
<td>93.3</td>
<td>63.0</td>
<td>92.9</td>
<td>72.7</td>
<td>30.8</td>
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<td>Chloramphenicol</td>
<td>0</td>
<td>66.7</td>
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<td>72.7</td>
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<td>3.6</td>
<td>27.3</td>
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<td>0</td>
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<td>Norfloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>ND</td>
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</tr>
</tbody>
</table>

ND, not determined.

By using XhoI-PFGE, 14 distinct profiles were found: 9 (X1–X9) in Son La province isolates and 5 (X10–X14) in CS (Table 3, Figure 2A). Profile X1 was the most frequently observed PFGE profile in PFGE-typed Son La province isolates (19/29, 65.5%), whereas it was not found in CS. Profile X2 differed from X1 by an additional low-molecular-weight band of ~70 kb, possibly corresponding to a plasmid. Profiles X3–X9 differed from X1 by one to four bands >100 kb. Clustering analysis performed by UPGMA revealed that Typhi isolates from Son La province clustered together (85% similarity) (Figure 2B). A significant genetic diversity was observed between the Son La province isolates and the CS.

Combination of the three classic molecular typing methods results indicated that IS1-344-X1 was the most frequently encountered combined profile in Son La province isolates (12/29, 41.4%). This combined profile was observed in outbreak or sporadic isolates collected from Son La town and from all the three districts of the study (Table 3).

By using the newly described haplotyping method, only one haplotype, H68 was obtained among the 31 isolates from Son La (Table 3). This haplotype was characterized by two synonymous single-nucleotide polymorphisms (sSNP) located in the genes HemD and fadD and by an insertion of 17 nucleotides in the gene STY2629, compared with the haplotype of reference CT18. Four additional haplotypes were found in the CS, the haplotype of 162/95 and 119/96 were unique whereas 230/95, 14/96, 358/98, and 339/98 harbored the same haplotype, H58.

**DISCUSSION**

The present study revealed that almost all of the Typhi isolates collected from four outbreaks and sporadic cases in Son La province, Vietnam, in 2002 were susceptible to classic first line antibiotics. It is an interesting finding because MDR and MDR-NalR Typhi isolates have been established in this province since at least 1995 and 1997, respectively. A decline of MDR Typhi isolates has been noted in India, whereas, in contrast to our study, isolates with a single resistance to nalidixic acid (and a decreased susceptibility to ciprofloxacin) were reported to increase. Throughout Vietnam, emergence of Typhi isolates that are resistant only to nalidixic acid has also been observed since 2002 (H. Le, unpublished data). The discrepancy between the trends of antimicrobial resistance in Son La province and other regions of Vietnam may be explained by differences in geography and access to health care. In Son La province, TF outbreaks were often restricted to small communities living in mountainous areas. Because of poor road quality (it can take 2 days to reach study sites from Son La town) and a very low average income, access to antibiotics is limited. In richer Vietnamese regions, antibiotics can be bought over the counter and self-medication is common.

The genetic relatedness of the Typhi strains isolated in Son La province in 2002 was assessed using phage typing and four molecular typing methods. All but four isolates from Son La in 2002 were of phage type A. The remaining isolates could not be typed (DVS and Vi negative). In a previous study, E1 (N = 38) and E3 (N = 24) were the most frequent phage types observed in 81 epidemiologically independent MDR Typhi isolates collected throughout Vietnam during 1995–
In another study, untypeable Vi (UVS) and E1 were susceptible to M3 IS2 26a X13 H50 – ACSulTpTe E1 IS2 236 X10 H58. Rather than suggesting that the ribotyping results were more difficult to interpret. One ribotype, 344, was found in 57.6% of the isolates. Seven isolates with a predominant PFGE profile, X1, showed ribotypes other than 344. For these isolates, we could not determine a phage type and subsequently disturb cluster analysis, ribotyping power of I-PFGE alone.

Haplotyping revealed that the strains isolated in Son La bore a unique haplotype, H68, which was characterized by combination of 2 sSNP located in the genes HemD and fadD and a 17-bp insertion located in the gene STY2629 (Figure 3). The HemD SNP was common among Vietnamese strains, whereas the 17-bp insertion was Son La-specific. This insertion was not present among 480 worldwide strains, including 149 susceptible MDR and MDR-Nal. Typhi strains isolated from several Vietnamese regions. In addition, XbaI-PFGE, considered the method of choice for subtyping Typhi, revealed that the Son La isolates were highly related with profiles clustering into the same group with a similarity of 85%. The ribotyping results were more difficult to interpret. One ribotype, 344, was found in 57.6% of the isolates. Seven isolates with a predominant PFGE profile, X1, showed ribotypes other than 344. For these isolates, we could have performed PFGE with another restriction enzyme to check if these isolates belonged to the same or to different clones. However, in studies involving Typhi, use of additional enzymes did not significantly enhance the discriminatory power of XbaI-PFGE alone. Rather than suggesting different clones, ribotyping results could be explained by possible homologous recombinations between rrm operons of related isolates, as previously described by Echeita and Usera. As these rearrangements can dramatically modify the ribotype and subsequently disturb cluster analysis, ribotyping
should be used with caution and concomitantly with other methods like PFGE during epidemiologic investigations.

It remains difficult to define whether the Son La clone had emerged from a local strain or had been imported. Only a single earlier isolate from Son La province was available for the present study (other isolates were not stored). This MDR-Nal\textsuperscript{R} isolate (14/96) collected in 1996 was characterized by a different haplotype, IS\textsuperscript{200}-type, and phage type in comparison with the 2002 clone. This 14/96 isolate was similar to 230/95, an MDR isolate collected in 1995 in Than Hoa province, located near Son La province. They both belong to the H58 haplotype, which is now found predominantly in Vietnam and South Asia in MDR and MDR-Nal\textsuperscript{R} Typhi strains.\textsuperscript{29} This suggests that the Son La susceptible clone did not emerge from a plasmid-purged MDR Typhi strain.

In the absence of earlier isolates collected in Son La province, we have no information on when exactly the clone emerged in this province. Molecular analysis performed in the past showed that, in contrast to MDR, susceptible Typhi strains displayed extensive genetic heterogeneity.\textsuperscript{35,41} The very weak genetic diversity of the Son La clone suggests that it might have emerged rather recently in Son La province.

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**Figure 1.** Representative IS\textsuperscript{200} profiles \textbf{A}, and \textit{PstI}-ribotypes \textbf{B}, obtained from the 33 selected Typhi isolates and the 8 comparison strains under study. \textbf{A}, Lanes 1–9, IS1 type; lane 10, IS2 type. \textbf{B}, Image generated by BioNumerics. M, Riboprinter marker size (band sizes in kilobase pairs). Ribotype numbering according to BBPE-Unit database is indicated. Asterisks indicate the ribotypes observed in isolates from Son La province, 2002.

**Figure 2.** \textbf{A}, Representative \textit{XbaI}-PFGE profiles obtained from the 29 typed Typhi isolates and the 8 comparison strains under study. M, \textit{S. enterica} serotype Braenderup H812 used as the molecular size marker (band sizes in kilobase pairs). PFGE profile numbering is indicated. \textbf{B}, Dendrogram generated by BioNumerics showing the results of cluster analysis on the basis of PFGE fingerprinting. Similarity analysis was performed using the Dice coefficient, and clustering was by UPGMA. Numbers in parentheses refer to the number of isolates with the indicated PFGE profile.
This susceptible clone could have been established in Son La province as early as 1997, when MDR isolates were reported to decrease, or as late as 2002. It could have been acquired before circulation of MDR or MDR-NalR Typhi isolates and maintained in chronic carrier(s) until favorable epidemiologic conditions associated with the disappearance of antibiotic selective pressure, leading to its dissemination in Son La province. In the first hypothesis, the circulation of this clone during the last decade might have resulted in a network of carriers harboring the same or a closely derived strain and being the source of small independent outbreaks in distinct communities. In the second hypothesis, the three outbreaks and the sporadic cases of 2002 were related. The previous epidemiologic study did not support this hypothesis. However, the possibility of a village-to-village dissemination during the 10-week period by healthy carriers was not investigated (only TF cases were investigated). Healthy carriers could have contaminated people through point source contaminated food or more probably through water from wells or streams. Drinking untreated water and poor hygiene habits were among the risk factors found to be associated with TF in the province (83/90 cases and 149/180 controls).22 Nevertheless, in the absence of earlier isolates, the cause(s) that should explain the Son La province scale replacement of the MDR/MDR-NalR Typhi strains by a single susceptible clone described herein remain(s) unclear. The Son La paradox sheds light on the possible shift from multiresistant populations to a susceptible population in one region where TF is already endemic. Understanding the epidemiologic situation that has led to this shift of populations in Son La would be very important for TF control.

The finding that four amoxicillin-resistant isolates have been detected among isolates belonging to the emerging or re-emerging clone(s) indicates that acquisition of resistance determinants is already started. Reasons for emergence of resistance are well documented and are most probably preventable. A resurgence of MDR and MDR-NalR Typhi isolates in Son La province, whatever their clonal lineage, should be prevented by the use of classic first-line antibiotics adapted to the results of the monitoring of antimicrobial susceptibility when feasible. Education of health professionals to ensure appropriate antibiotic prescriptions and education of patients to avoid self-medication should be emphasized.

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