Endemic, Epidemic Clone of *Salmonella enterica* Serovar Typhi

Thi Anh Hong Le, Monique Lejay-Collin, Patrick A. D. Grimont, Thuy Long Hoang, Thi Vinh Nguyen, Francine Grimont, and Maurice R. Scavizzi

Laboratoire d’Épidémiologie de la Résistance Bactérienne, Institut National d’Hygiène et d’Épidémiologie, University of Medicine of Hanoi, Hanoi, Vietnam, and Unité de Biodiversité des Bactéries Pathogènes Emergentes, U389 INSERM, Institut Pasteur, and Faculté de Santé, Médecine et Biologie Humaine de Bobigny, Université Paris Nord, Paris, France

Received 22 October 2003/Returned for modification 30 December 2003/Accepted 13 April 2004

*Salmonella enterica* serovar Typhi strains resistant to ampicillin, chloramphenicol, tetracyclines, streptomycin, and cotrimoxazole, isolated from sporadic cases and minor outbreaks in Vietnam between 1995 and 2002, were typed and compared. Plasmid fingerprinting, Vi bacteriophage typing, XbaI pulsed-field gel electrophoresis, and PstI ribotyping showed that endemic, epidemic multidrug-resistant typhoid fever was due, for at least 74.1% of the isolates, to one or two clones of serovar Typhi harboring a single resistance plasmid. PstI ribotyping was used as a basic technique to ensure that a serovar Typhi expansion was clonal.

In many developing countries, extended local outbreaks of typhoid fever occur against a background of sporadic cases. For instance, in 1972 the endemic form turned Epidemic in southern Vietnam (22). Moreover, resistance of *Salmonella enterica* serovar Typhi to chloramphenicol appeared in this country at the end of 1971. During the following years, resistant strains have been increasing isolated (211 resistant strains of 288 isolates between 1972 and 1975, i.e., 73.3%, reaching 85.4% in 1975). These strains were found to be multidrug-resistant (MDR), i.e., resistant to chloramphenicol, tetracyclines, streptomycin, and sulfonamides. Resistance was encoded by self-transferable plasmids (22, 39). Several studies have been published about typhoid fever and MDR strains worldwide, but none from Vietnam was published until the beginning of the 1990s; during this time, serovar Typhi isolates became susceptible again in Vietnam, with a few MDR strains (Le Thi Anh Hong, personal communication). In 1993, an epidemic of typhoid fever in the Kien Giang province (southern Vietnam) was again due to MDR isolates, i.e., resistant to ampicillin, chloramphenicol, tetracyclines, and trimethoprim-sulfamethoxazole (cotrimoxazole) (28, 34). MDR strains have been increasing, accounting for over 80% of all isolates by 1994 in southern Vietnam (49). At this time, less than 5 and 10% of MDR strains were isolated in the center and the north of Vietnam, respectively (30, 35). In and after 1995, over 90% of the isolates from both regions were MDR with the previously observed resistance pattern; in particular, an outbreak in 1996 in Hue Province consisted of MDR strains (29). Finally, since 1996, strains resistant to quinolones were more and more frequently isolated in Vietnam, and most of them were MDR (51).

It is useful to understand the mode of spread of serovar Typhi strains in order to implement rational strategies for the prevention of typhoid fever. Is it caused by multiple serovar Typhi clones or a single one? For this purpose, several methods have been used. For instance, in southern Vietnam, Wain et al. (50), using pulsed-field gel electrophoresis (PFGE), bacteriophage typing, and ribotyping, described a variety of different serovar Typhi clones in sporadic typhoid cases.

The aim of this study was to determine whether the endemic, epidemic MDR typhoid fever found throughout Vietnam from 1995 to 2002 was due mainly to a single resistance plasmid and to a single serovar Typhi clone or not. The answer can be useful to indicate suitable measures in the field of public health. We picked epidemiologically independent isolates, performed restriction typing of plasmids, and compared serovar Typhi strains by phage typing and ribotyping.

MATERIALS AND METHODS

Bacterial strains, susceptibility tests, and flow chart of strains and methods. Between 1995 and 2002, 519 *S. enterica* serovar Typhi clinical isolates were collected throughout Vietnam in the Laboratoire d’Épidémiologie de la Résistance Bactérienne (ERB-lab), Institut National d’Hygiène et d’Épidémiologie (INHE), Hanoi, Vietnam, i.e., 363 isolates during the period from 1995 to 1997 and 156 from 1998 to 2002. Four hundred twenty-six of the strains collected were MDR: 194 from the north, 116 from the center, and 116 from the south; 78.7% were MDR during the period from 1995 to 1997, and 89.9% were MDR during 1998 to 2002. One hundred forty-two MDR and 18 susceptible serovar Typhi strains, epidemiologically independent, were selected and studied. They were identified with the API 20E system (BioMérieux, Marcy l’Étoile, France). Agglutination was performed with antisera specific for the O:9, Vi, and H:sl antigens (Bio-Rad, Hanoi, Vietnam). Susceptibility tests were carried out by the disk (Bio-Rad) diffusion method and interpreted according to published standards and the comparative interpretative reading method (42, 43). The latter is based on multivariate data analyses which separate the different bacterial phenotypes of susceptibility and resistance, generated by genetic determinants and biochemical mechanisms; this approach is especially relevant for work on the epidemiology of antimicrobial resistance, which is our purpose.

Antibiotics implicated in the resistance of serovar Typhi isolates were tested, i.e., ampicillin, chloramphenicol, tetracycline, cotrimoxazole, nalidixic acid, and streptomycin (not in therapy now).
Eleven of these 81 ribotyped MDR isolates were tested by PFGE. Three MDR strains isolated from 1974 to 1975 in Saigon (now Ho Chi Minh City), Vietnam, were added to the study.

The reference strains were E. coli R39 harboring four plasmids of different sizes, 98, 46, 24, and 4.6 MDa, kindly supplied by the Laboratory of Bacteriology, Center for Tropical Diseases, Ho Chi Minh City, Vietnam; E. coli K-12 35-3, lactose positive, resistant to rifampin, kindly supplied by the Laboratoire de Bacteriologie, Hôpital Universitaire Avicenne, Bobigny, France; and E. coli W3110, lactose positive, resistant to nalidixic acid, kindly supplied by the Department of Bacteriology, University of Medicine of Hanoi, Hanoi, Vietnam.

**Conjugational transfer of drug resistance.** Conjugational transfers were carried out in ERB-lab, INHE, Hanoi, from 142 selected MDR serovar Typhi isolates to plasmid-free strains of both rifampin-resistant and nalidixic acid-resistant E. coli. All mating procedures were performed at 37°C for 18 h before plating onto Drigalski medium (Bio-Rad) containing 20 mg of ampicillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The other patterns (13 of 142 isolates) differed by one or several resistance markers; they were resistant to ampicillin and ticarcillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The other patterns (13 of 142 isolates) differed by one or several resistance markers (Table 1). More and more isolates have expressed resistance to nalidixic acid and fluoroquinolones since 1996.

**RESULTS**

**Resistance phenotypes and transfer of resistance plasmids.** All the resistance-related antibiotics had MICs of between 128 and >512 mg/liter for the MDR serovar Typhi isolates and their E. coli transconjugants.

Most MDR isolates (129 of 142) exhibited the same resistance pattern; they were resistant to ampicillin and ticarcillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The other patterns (13 of 142 isolates) differed by one or several resistance markers (Table 1). More and more isolates have expressed resistance to nalidixic acid and fluoroquinolones since 1996.

**Resistance phenotypes and transfer of resistance plasmids.** All the resistance-related antibiotics had MICs of between 128 and >512 mg/liter for the MDR serovar Typhi isolates and their E. coli transconjugants.

Most MDR isolates (129 of 142) exhibited the same resistance pattern; they were resistant to ampicillin and ticarcillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The other patterns (13 of 142 isolates) differed by one or several resistance markers (Table 1). More and more isolates have expressed resistance to nalidixic acid and fluoroquinolones since 1996.

All but five of the MDR isolates transferred en bloc the multidrug resistance (mainly ampicillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole) to rifampin- or nalidixic acid-resistant E. coli. Resistance to nalidixic acid and fluoroquinolones was never transferred to...
rifampin-resistant *E. coli*. The frequency of transfer ranged from $10^{-8}$ to $10^{-5}$ drug-resistant conjugants per donor cell. All but 15 of the *E. coli* transconjugants expressed the same resistance pattern as that of the serovar Typhi donors. The other 15 transconjugants expressed resistance patterns which differed by one or several resistance markers.

The main resistance pattern differed from the phenotype exhibited in 1974 to 1975 (which included resistance to chloramphenicol, tetracycline, streptomycin, and sulfonamide) by acquiring new resistance characters probably related to new drugs without losing the previous ones even though these resistance-related drugs are no longer used.

**Plasmid profile analysis.** The 107 MDR serovar Typhi isolates investigated and their transconjugants harbored a large plasmid (184.8 ± 8.2 kbp, i.e., ~122 MDa), and 50 of these serovar Typhi isolates contained a second plasmid (94.6 ± 3.5 kbp, i.e., ~63 MDa); this second plasmid was not always transferred to *E. coli* (Fig. 1). The transconjugants which harbored the single 122-MDa plasmid were MDR.

Some other serovar Typhi isolates which harbored the single 63-MDa plasmid were susceptible to antibiotics. Incidentally, a few serovar Typhi isolates were recognized as MDR at the moment of isolation, but after conservation, they were found not to carry any 122-MDa plasmid; we retested these strains and recognized them as being susceptible to antibiotics. Therefore, multidrug resistance was associated with a 122-MDa self-transferable plasmid.

**Vi phage types.** Vi phages types E1 and E3 were predominant among the MDR serovar Typhi isolates (62 of 81 strains, i.e., 76.5%) (Table 1). Since 1995, most isolates from the north exhibited phage type E1 and only one showed the E3 type (1999); isolates that originated from the center had mainly phage type E3 and one had the E1 type (1996); isolates from the south were a mixture of both phage types from 1995 to 2002. Nine of the 81 isolates studied (11.1%) were phage untypeable (Vi−), and four isolates that were Vi positive were not sensitive to the phages used. Other types definitely different from the E types were observed for six isolates between 1995 and 2002, i.e., phage types M4, D4, and 46.

Eighteen serovar Typhi isolates susceptible to antibiotics and harboring no plasmid or only a 63-MDa plasmid exhibited various phage types, i.e., A, M2, M3, and also E1 or E3, or were not sensitive to the phages used.

The three strains isolated in 1974 to 1975 in Saigon were Vi− or had the phage type 38, not found in the present study.

**Ribotypes.** Ribotype 3a (five bands) was associated with 79 of 81 (97.5%) MDR isolates (Table 1, Fig. 3). Two other ribotypes isolated in 1995 in the north were 230 and 236, associated with phage type E1. Therefore, ribotype 3a associated with phage types E1 and E3 was observed for 60 of 81 MDR serovar Typhi isolates, i.e., 74.1%. All nine phage-untypeable (Vi−) isolates exhibited ribotype 3a.

Among the 18 isolates susceptible to antibiotics, 10 exhibited various ribotypes, 3ab, 26a, 187, 228, 237, 240, and 241; eight were 3a, seven of these exhibiting phage type E1 or E3, which suggests loss of the large resistance plasmid.

All these plasmids were identical with respect to both restriction digests.

**FIG. 1.** Electrophoresis on an agarose gel (0.7%, 80 V, 3 h) of plasmid DNA extracted from MDR serovar Typhi isolates and *E. coli* transconjugants. Lanes 6 and 16, four plasmids (98, 46, 24, and 4.6 MDa) from reference strain *E. coli* R39; lane 15, no plasmid from reference strain *E. coli* K12 J5-3; lanes 1, 3, 11, and 13, both 122- and 63-MDa plasmids from isolates; and lanes 2, 4, 5, and 12, both 122- and 63-MDa plasmids from their transconjugants; lanes 7 and 8, only the 122-MDa plasmid from an isolate and its transconjugant, respectively; lane 10, both plasmids from an isolate; and lane 9, only the 122-MDa plasmid from its transconjugant; lane 14, the single 122-MDa plasmid from an isolate. (A) Photograph of the agarose gel. (B) Image analyzed by computer.

**FIG. 2.** Electrophoresis on an agarose gel (0.8%, 40 V, 16 h) of HindIII restriction digests of 122-MDa plasmid DNA extracted from *E. coli* transconjugants of MDR serovar Typhi isolates. Lane 1, HindIII-digested bacteriophage lambda DNA. (A) Photograph of the agarose gel. (B) Image analyzed by computer.
The three MDR strains isolated in 1974 to 1975 in Saigon had various ribotypes, 151, 239, and 263. **PFGE fingerprints.** The number of DNA fragments obtained by XbaI digestion ranged between 11 and 13 (results not shown). Among the 11 isolates of ribotype 3a which were PFGE typed, four patterns were observed. PFGE pattern 1 was shown by three phage type E1 isolates from north and south Vietnam and five phage type E3 isolates from central and south Vietnam. PFGE pattern 2 was shown by one phage type E1 isolate, PFGE pattern 3 was shown by one phage type E1 isolate, and PFGE pattern 4 was shown by one phage type E3 isolate. PFGE patterns 2 to 4 differed from PFGE pattern 1 by only two or three fragments.

**DISCUSSION**

After the MDR typhoid fever epidemic occurred in Mexico during 1972 (31), large autotransferable resistance plasmids harbored by MDR serovar Typhi strains have been isolated worldwide, mainly in developing countries where typhoid fever remains endemic: South America (13), Africa (1), the Middle East (33), southern Asia (2, 17, 19, 23, 40, 48), and southeast Asia (20, 21, 36). Most of these plasmids but not all belonged to the H1 incompatibility group, but their molecular size varied between 73 and 192 MDa; the size could differ among plasmids obtained from the same epidemic and from one country to another but was stable during every outbreak; in particular, an estimated 140-MDa plasmid was harbored by nearly all the MDR isolates from southern Vietnam between 1993 and 1997 (6). Therefore, the incompatibility grouping of plasmids is a useful epidemiological tool (5, 7, 10) but is less precise than restriction pattern analysis. The restriction patterns obtained for the 122-MDa plasmids were identical for all the strains studied. We used two different endonucleases, not a single one, to confirm plasmid identity. We can conclude that the MDR serovar Typhi strains isolated throughout Vietnam between 1995 and 2002 harbored a single resistance plasmid. Has a strain which harbored this plasmid taken advantage of this fact to achieve a clonal expansion?

The human-adapted serovar Typhi was considered by Se-lander et al. (44) to be one of the least genotypically heterogeneous Salmonella serovars; however, they pointed out considerable variation in its rRNA operon. Boyd et al. (4) investigated the genomic content of a set of isolates: they found such differences that they concluded the genomic reservoir was unstable, even within a highly clonal bacterial population. Several workers using ribotyping with or without PFGE (3, 11, 12, 16, 26, 27, 32) demonstrated that PstI ribotyping is a reliable technique, i.e., stable, reproducible, and sensitive for subtyping serovar Typhi. PstI ribotyping displays a high discriminatory power even if it can only detect changes at restriction sites at different copies of the rRNA gene, whereas differences in restriction sites at regions other than the ribosomal operon could be detected by digestion of chromosomal DNA (21).

A good correlation was established between the heterogeneous patterns obtained from both XbaI PFGE and PstI ribotyping of isolates involved in sporadic cases (46). The results of Ling et al. (21) (95 and 124 patterns obtained by PstI ribotyping and Nal PFGE, respectively, from 290 isolates), those of Mirza et al. (24) (five patterns divided into 11 subgroups from 193 MDR isolates with XbaI PFGE) and those from Thong et al. (47) (11 of 23 paired isolates from individual patients exhibited genetic changes with XbaI PFGE) lead us to estimate that the higher discriminatory power of PFGE displays clonal variations more than independent clones, whereas the PstI ribotyping is a solid tool to separate real clones; thus, Pang et al. (32) separated eight clones from 20 isolates by PstI ribotyping. Moreover, ribotyping could not only recognize homogeneous clones related to outbreaks, but also discriminate isolates from sporadic cases (26, 27).

The results of the present study are in accord with these works. Eight isolates were indistinguishable by PFGE. For three isolates, two or three band variations in PFGE patterns are not epidemiologically significant. According to Tenover et al. (45), all isolates of ribotypes 3a that were PFGE typed were closely related irrespective of their classification as phage type E1 or E3.

From 1997 to the end of 2003, 1,154 serovar Typhi strains had been collected, phage typed, and ribotyped and were differentiated into 302 ribotypes in the BBPE-Unit (16). In this serovar Typhi strain collection, Vi phage types E1 and E3 represent 16 and 0.7%, respectively; ribotype 3a represents 11.3%; and the associated phage type E1-ribotype 3a represents 5.5%. These figures indicate that ribotyping in combination with phage typing had high discriminatory power and so is an accurate epidemiological tool, in any case for S. enterica serovar Typhi. The use of both methods appears suitable to determine whether serovar Typhi isolates belong to the same clone or not.

Our results have shown that the association of ribotype 3a with phage type E1 or E3 was strongly predominant (74.1%) among MDR serovar Typhi isolates collected throughout Vietnam from 1995 to 2002, while the phage types of MDR serovar Typhi strains isolated in 1974 to 1975 in Saigon were different. We can assume that most of the MDR typhoid fever cases which occurred over 8 years in Vietnam were due to the clonal expansion of one or two strains, depending on whether the distinction between Vi phage types E1 and E3 is reliable or not. If the distinction is reliable, two predominant clones have
coexisted in the south, at least in Ho Chi Minh City and in three provinces, since 1995 and even before (6), phage type E1 strongly predominating in the north and phage type E3 in the center.

If the distinction between phage types E1 and E3 is not reliable, and taking into account a proportional part of the Vi-untypable strains, all harboring the 3a ribotype, most MDR serovar Typhi isolates (four-fifths) exhibited genetic homogeneity. This means that the endemic, epidemic MDR typhoid fever in Vietnam was due for the most part to a single clone spreading from the south to the center and the north from 1995 to 2002. This is supported by results from the BBPE-Unit collection (Institut Pasteur, Paris): among ribotype 3a strains, phage types E1 and E3 represent 49.2 and 0%, respectively; of 29 3a/E1 serovar Typhi isolates with travel history, 20 were associated with travel in Asia (India, Pakistan, and Laos). Moreover, serovar Typhi type E3 is less susceptible to Vi bacteriophages than type E1. Thus, clone 3a-E1, found in Vietnam as well as in Asian countries, could have evolved in Vietnam into a new clone, 3a-E3, which was never seen in our collection before. Therefore, one clonal expansion (one ribotype) which evolved from one phage type to another and underwent limited chromosomal rearrangements (pointed out by PFGE fingerprints) is a valid hypothesis.

In countries where typhoid fever is endemic, isolates from the same outbreak appeared to be genetically identical, whereas sporadic cases were caused by a wide variety of types (6, 11, 12, 17, 21, 27, 32, 46). We detected various phage types and ribotypes among the serovar Typhi isolates from sporadic cases, but only among the antibiotic-susceptible ones. In contrast, the results from plasmid fingerprinting, phage typing, and PstI ribotyping show that most of the MDR serovar Typhi strains isolated from both sporadic cases and minor outbreaks over 8 years throughout Vietnam, and of course from the epidemic which occurred in Hue in 1996, belonged to one or at the most two clones and harbored a single resistance plasmid.

The outcome of our study will lead to the implementation of rational strategies and suitable measures in the field of public health in order to prevent MDR typhoid fever, insofar as clonal spreading of MDR S. enterica serovar Typhi is likely to be due to a network of carriers rather than to multiple sources of infection.

ACKNOWLEDGMENTS

Thanks are due to Nguyen Thi Kim Hoang, Institut Pasteur, Ho Chi Minh City; Tran Huu Luyen, Hôpital Central, Hœœ; Doan Mai Phuong, Center for Infectious Diseases, Hanoi; Nguyen Thi Mai Hoa, Hôpital Saint Paul, Hanoi; and Ngo Thi Thi, Hôpital Pédia-trique, Hanoi, Vietnam, for providing strains. We thank To Song Diep and John Wain, Center for Infectious Diseases, Ho Chi Minh City, for stimulating discussions. We thank Luong Ngoc Tram and Nguyen Thi Than Ha, Institut National d’Hygiène et d’Épidémiologie (INHE), Hanoi, for checking the sera and testing the antibiogram susceptibility of strains. The technical assistance of Corinne Ruckly, Unité de Biodiversité des Bactéries Pathogènes Emergentes, Institut Pasteur, Paris, of Luong Diem Nga, Center for Infectious Diseases, Ho Chi Minh City, and of Tran Thi Hai Au, Le Lan Huang, and Nguyen Thi Hien Anh, INHE, Hanoi, is gratefully acknowledged. Encouragement from J.-L. Durosier and J. M. Larray is much appreciated.

This work was supported by Délégation Générale au Réseau International des Instituts Pasteur et Instituts Associés and Université Paris Nord, Bureau des Relations Internationales.

REFERENCES

as a tool in epidemiological analysis of Salmonella typhi infections. Epide-
27. Navarro, F., T. Llovet, M. A. Chepita, P. Coll, A. Aladuen, M. A. Usera, and
resistance of 363 strains of Salmonella typhi isolates collected from patients
Vietnamese.)
isolates collected from Central Hospital in Hue during 1983–1993. Studies on
97–102. Center for Medical Information, Hanoi, Vietnam. (In Vietnamese.)
31. Olarte, J., and E. Galindo. 1973. Salmonella typhi resistant to chlorampheni-
col, ampicillin, and other antimicrobial agents: strains isolated during an
Genetic variation among Malaysian isolates of Salmonella typhi as detected
543.
1:439–442.
34. Pham, K. S. 1995. From an outbreak of typhoid fever in the District An Minh
(In Vietnamese.)
pathogenic bacteria that leads to blood infection at the Hospital Bach Mai
on 1993. Studies on antimicrobial susceptibility of pathogenic microorgan-
(In Vietnamese.)
and conjugative chloramphenicol and tetracycline resistance plasmids in
method using a chemically labeled oligonucleotide probe mixture. Res. Mi-
method using a chemically labeled oligonucleotide probe mixture. Res. Mi-
crobiol. 149:73.
souches de salmonelles isolées de 1961 à 1975 à l’hôpital Grall de Saigon.
laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, N.Y.
42. Scavizzi, M. R., A. Elbhar, J. P. Fenelon, and F. D. Bronner. 1993. Multi-
dimensional analysis for interpreting antibiotic susceptibility data. Anti-
susceptibility test: from bacterial population analysis to therapy. Int. J.
Kopeco, K. Ferris, B. D. Tall, A. Cravioto, and J. M. Musher. 1990. Evo-
lutionary genetic relationships of clones of Salmonella serovars that cause
45. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray,
restriction patterns produced by pulsed-field gel electrophoresis: criteria for
Epidemiologic analysis of sporadic Salmonella typhi and those from out-
47. Thong, K. L., Y.-L. Goh, R. M. Yasun, M. G. Lau, M. Passey, G. Winston,
M. Yoannes, T. Pang, and J. C. Reeder. 2002. Increasing genetic diversity of
Salmonella enterica serovar Typhi isolates from Papua New Guinea over the
48. Threlfall, E. J., L. R. Ward, B. Rowe, S. Raghupathi, V. Chandrasekaran, J.
11:990–993.
49. Vinh, H., J. Wain, T. N. Vo, N. N. Cao, T. C. Mai, D. Bethell, T. T. Nguyen,
S. D. Tu, M. D. Nguyen, and N. J. White. 1996. Two or three days of oxolinic
acid treatment for uncomplicated multidrug-resistant typhoid fever in children.
Dougan. 1999. Molecular typing of multiple-antibiotic-resistant Salmonella
enterica serovar Typhi from Vietnam: application to acute and relapse cases
resistant Salmonella typhi in Viet Nam: molecular basis of resistance and
ERRATUM


Thi Anh Hong Le, Monique Lejay-Collin, Patrick A. D. Grimont, Thuy Long Hoang, Thi Vinh Nguyen, Francine Grimont, and Maurice R. Scavizzi

Laboratoire d’Épidémiologie de la Résistance Bactérienne, Institut National d’Higiène et d’Épidémiologie, and University of Medicine of Hanoi, Hanoi, Vietnam, and Unité de Biodiversité des Bactéries Pathogènes Emergentes, U389 INSERM, Institut Pasteur, and Faculté de Santé, Médecine et Biologie Humaine de Bobigny, Université Paris Nord, Paris, France

Volume 42, no. 7, p. 3094–3099, 2004. Page 3097, column 1, Discussion. Lines 7–11 should read as follows: “... Most, though not all, of these plasmids belonged to the H1 incompatibility group, but their molecular size varied between 73 and 192 MDa, with plasmids of various sizes being obtained from different epidemics and from different countries, though plasmid size tended to remain stable during any given outbreak. In particular, ...”