Molecular Typing Reveals a Unique Clone of *Salmonella enterica* Serotype Typhi among Indian Strains

Typhoid fever (TF) is endemic in India, and a seasonal upsurge of cases is observed annually. Although mortality from TF has been greatly reduced by effective therapies and the availability of vaccines, morbidity is still high due to the global emergence of multidrug resistance and subsequent treatment failures (4). Studies have also documented the circulation of multiple genetic variants (17) and various clinical manifestations of TF (5). In an ongoing study comprising molecular subtyping of *Salmonella enterica* serotype Typhi strains collected over the past 5 years, we encountered a strain cluster that had been isolated during a seasonal upsurge of TF cases. A total of 16 strains was recovered serially from cases reported during a week's time. We highlight the emergence of a unique clone of serotype Typhi strains circulating in the Delhi region.

Among the probes used for strain typing with DNA fingerprint analysis, insertion sequences (IS) have acquired great popularity (7). While originally discovered in the genome of serotype Typhimurium LT2, one such shorter element, IS200, a 707- to 710-bp sequence, is widely distributed among conserved loci of the chromosomes of most *Salmonella* serotypes (7, 10). The apparent copy numbers, 4 to 7 in serotype Paratyphi A and 10 to 25 in serotype Typhi, and identities among strains in the actual copy numbers and positions of IS200 indicate their evolutionary origin (6, 15). Since IS200, unlike other entrobacterial IS elements (e.g., IS1 and IS5), rarely translocates under laboratory conditions, its use has become increasingly popular for strain differentiation, including the determination of the origins of drug-resistant strains (18). Studies with sufficiently large numbers of strains have clearly shown that IS200 profiles are indeed characteristic for the serovars, where the patterns are not only of value as epidemiological markers but also sufficiently stable to allow reconstruction of molecular phylogenies (2).

In the present study, salmonella-specific IS200 fingerprinting (15) and pulsed-field gel electrophoresis (17) were used in the characterization of serotype Typhi strains from this identified episode. Genome hybridization by IS200 probing revealed two distinct profiles: insertion sequence type I (IST-I) and IST-II. The IS200 probe traced either 11 to 14 hybridization bands (IST-I) or a mere 2 hybridization bands (IST-II) bearing IS200 insertions distributed over PstI-digested DNA fragments ranging from 1 to 25 kb in size. IST-II represented a unique pattern, as revealed by the presence of 2 IS200-probed bands versus the 10 to 25 apparent copies consistently observed for the global population of serotype Typhi strains. Six out of the 16 strains were assigned this unique pattern (Fig. 1).

PFGE analysis using XbaI showed two major profiles: pulsed-field profile I (PFP-I), with 14 to 17 genomic fragments ranging from 550 kb to 20 kb, and PFP-II, showing 11 to 12 fragments of 440 kb to 20 kb. Notably, major differences in genome sizes were observed among PFPE types. The absence of nearly 500 to 850 kb of the genomic portion of PFP-II resulted in reduced genome sizes ranging between 3.7 and 4.1 Mb, in comparison to the 4.4- to 4.8-Mb genomes observed among predominant serotype Typhi strains. Displaying a high degree of correlation in their discriminatory abilities, both the IS200 and the PFGE analyses discerned unique clones (IST-II and PFP-II) that were nearly identical (Fig. 2).

Clinically, the patients presented with the usual TF symptoms and without any significant complications. Overall, the strains did not reveal any significant phenotype variations. Based on phage group determination, the majority of the isolates grouped into a single phage type, E1; however, two strains from the unique cluster remained untypeable by available phage panels. Although several phage types linked to human disease activities have been recognized for serotype Typhi, one or a few types dominate within a geographical region for a specified period, presumably due to the spread of a successful clone or to the infrastructure of the host population (16).

A long-standing and paradoxical trait of IS200's behavior is its extremely low transposition frequency, which can be viewed as a self-restraint strategy imparting evolutionary advantages (1). Only a few cases of IS200 transposition or other forms of rearrangement have been documented in salmonellae (8). For instance, serotype Typhimurium strain LT2 was shown to harbor 6 copies of the IS200 element (7), whereas recent studies have actually evidenced 7 to 16 copies (15). Recently, a new clonal line of *Salmonella* serotype Saintpaul which virtually lacks this IS element has also been reported to be circulating in Japan (9).

It has previously been shown that the apparent number of serotype Typhi IS200 insertions ranges from 10 to 25 (7), while PstI profiling probed at least 11 to 13 bands (18). Recently, the whole-genome sequence comparisons of the serotype Typhi Ty2 and CT18 strains have actually revealed 26 copies of IS200 (6); however, earlier IS200 profiling of PstI-digested genomic DNA of the same strains yielded only 15 discrete hybridization signals (3). These studies point towards a remarkable conservation of numbers and locations of the IS200 element among serotype Typhi strains globally and also indicate that the number of hybridization fragments may not depict the actual copy number, since individual bands of IS200 profiles either may carry multiple copies of IS200 or may represent various copies over PstI fragments of similar sizes. In the present study, the new unique pattern (IST-II) showed only 2 apparent IS200 hybridizing bands compared to 11 to 14 copies consistently present among the majority of Indian serotype Typhi strains.

The findings suggest that recent genomic rearrangements may have caused the emergence of newer IS200 types during an outbreak in this region. It is well known that even a few genetic changes can generate large differences in pattern or number of hybridization bands displaying an uneven distribution of IS200 insertions. Therefore, caution should be exercised in the analysis of hybridization results, especially while probing a low-copy-number IS element, since it can affect the cluster analysis by separating otherwise closely linked strains.

Large-scale evolutionary genome alterations may affect the microbial ecology of this important human pathogen. Genomes of serotype Typhi are shown to undergo rearrangements due to homologous recombination among the seven copies of *rrn* genes (13). Therefore, even closely related strains may differ widely in gene content. Although most *Salmonella* serovars share over 90% genome homology, they often display drastically different properties per-
taining to host range specificity and the nature of the illnesses they cause. A recent study indicated that genomic regions encoding phenotypic features required in drug resistance and virulence mechanisms tend to evolve faster than other genomic regions due to diversifying selection pressure exerted by the host’s immune response (11).

Previously, seven strains of serotype Typhi have been shown to exhibit precise deletions of the entire 134 kb of salmonella pathogenicity island 7 (SPI7), all with a reduction in size of the I-CeuI (intron-encoded endonuclease) G fragment known to contain the SPI7-harboring gene for VI polysaccharide synthesis. Such alterations are believed to render many strains nonagglutinable by antiserum to VI antigen (12). Since, in the present study, two of six IST-II strains remained untypeable by Vi-phage grouping, these strains probably had asymmetrical genomic rearrangements. Notably, even large alterations have not substantially altered the stability or survival of this clone. This is perhaps not surprising in light of the extensive genomic diversities observed for the highly plastic serotype Typhi genomes, an intrinsic characteristic of intracellular bacteria. Whole-genome analysis of a strain with a multidrug resistance phenotype, CT18, revealed hundreds of insertions and deletions in size of the I-CeuI (intron-encoded endonuclease) G fragment, showing precise deletions of the entire 134 kb of SPI7, all with a reduction in size.

References


FIG. 1. Chemiluminescence X-ray signals generated by IS200 probing. Lanes 2 to 7, the unique IST-II pattern shared by six strains with two IS200-probed DNA fragments; lanes 1 and 8 to 16, the IST-I pattern shows a serotype Typhi-specific pattern with 11 to 14 apparent copies. Lanes A and B, known serovars Paratyphi A (A) and Paratyphi B (B) with six and five probed fragments, respectively, are included as controls. Notably, the two hybridized DNA fragments shown by the arrows are consistent in all serotype Typhi strains. M, molecular size standards.

FIG. 2. Comparative cluster analysis of IS200 and PFGE profiles. (A) The unique IST-II pattern (strains A2 to A7) revealed reduced apparent IS200 copies. The IST-I cluster shows a serotype Typhi-specific predominant pattern and further subtypes Ia to Ic. Serotype Paratyphi A (SPA) and serotype Paratyphi-B (SPB), included as controls, form a separate cluster with distinct band patterns. (B) PFGE also identified strains included in IST-II as a separate clone, PFP-II. Large genome alterations were revealed in PFP-II, the unique cluster.

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