Multidrug-Resistant Strains of *Salmonella enterica* Serotype *typhi* Are Genetically Homogenous and Coexist with Antibiotic-Sensitive Strains as Distinct, Independent Clones

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**ABSTRACT**

**Objective:** The goal of this study was to report the molecular analysis of antibiotic-sensitive and multidrug-resistant (MDR) strains of *Salmonella typhi*, using pulsed-field gel electrophoresis (PFGE), with a particular emphasis on the coexistence of these strains in a typhoid-endemic region of Karachi, Pakistan.

**Methods:** One hundred isolates of *S. typhi* in humans (50 MDR and 50 antibiotic-sensitive isolates) from sporadic cases of typhoid fever were analyzed by Vi-phage typing, antibiograms and PFGE.

**Results:** The MDR *S. typhi* strains were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Analysis by PFGE showed that 50 MDR isolates of *S. typhi* had a single, homogenous PFGE profile, which was distinctly different from that of 50 antibiotic-sensitive isolates obtained in the same time frame from the same area. This latter group of isolates showed much greater diversity of PFGE profiles, as has been observed in other endemic regions.

**Conclusions:** Multidrug-resistant and antibiotic susceptible strains of *S. typhi* can coexist in endemic areas as epidemiologically independent pathogens and are not in competition for continued persistence and transmission.

Key Words: antibiotic resistant, MDR Salmonella typhi, molecular typing, PFGE


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**MATERIALS AND METHODS**

One hundred isolates of *S. typhi* in humans obtained from blood were used in this study. These isolates were obtained from sporadic cases of typhoid fever occurring in patients admitted to the pediatric unit of the Aga Khan University Medical Center in Karachi, and consisted of 50 antibiotic-sensitive and 50 MDR isolates. The isolates were obtained between March 1996 and April 1997 from...
100 patients (58 males; 42 females) who ranged in age from 18 months to 14 years. Strains were isolated, identified, and maintained using standard methods at the collecting center (Aga Khan University Medical Center). Antibiotic sensitivity tests also were done by the collecting center, using the standard Kirby-Bauer disk-diffusion method. Phage typing of the strains was performed at the Salmonella Reference Center, Institute for Medical Research, Kuala Lumpur. Plasmid presence was determined by PFGE of undigested chromosomal DNA and by the alkaline lysis procedure of Kado and Liu. Genomic DNA was prepared by the method previously described. Isolates were analyzed by PFGE following digestion of chromosomal DNA with the restriction endonuclease XbaI (5'-TCTAGA-3'), according to previously published procedures. The preparation of DNA from strains was repeated, digested, and electrophoresed on at least two occasions, to assess the reproducibility of the method. Pulsed-field gel electrophoresis profiles were assigned arbitrary designations and analyzed by defining a similarity (Dice) coefficient:

\[
F = \frac{2n_{xy}}{n_x + n_y},
\]

where \(n_x\) = number of fragments for isolate \(x\),
\(n_y\) = number of fragments for isolate \(y\),
\(n_{xy}\) = number of fragments shared between isolates \(x\) and \(y\).

By this assessment, \(F = 1.0\) indicates complete pattern identity and \(F = 0.0\), complete dissimilarity.

RESULTS

The MDR isolates were resistant to chloramphenicol (30 µg), ampicillin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.5 µg). The majority (>80%) of the MDR strains of \(S. typhi\) belonged to phage type E1, whereas the antibiotic-sensitive strains had a variety of other phage types, including 45% untypeable Vi strain (UVS) 46 (16%), E1 (10%), and J1 and J5 (10%). Analysis by PFGE of these \(S. typhi\) isolates following digestion of chromosomal DNA with XbaI gave stable and reproducible DNA fragment patterns that consisted of between 14 and 22 bands (Figure 1). The antibiotic-sensitive isolates gave multiple PFGE patterns following XbaI digestion (37 different patterns among 50 isolates) that were distinctly different from one another, with differences in 2 to 12 bands (see Figure 1, \(F = 0.58\) to 1.00). In contrast, the MDR isolates showed essentially only a single PFGE pattern, with 1 of 50 isolates differing by a single band (Figure 2, \(F = 0.98\) to 1.00). Comparison of the sensitive and MDR isolates gave an F-value of 0.58 to 0.98. Plasmid analysis of the MDR isolates showed the presence of a plasmid with an approximate molecular mass of 120 MDa (data not shown). It also was noted that the single PFGE pattern detected among the MDR isolates also was present in 3 of 50 sensitive isolates, but that pattern differed by a single DNA fragment (see Figure 1, lanes 1-3 and Figure 2).

DISCUSSION

The data obtained in this study concur with previous observations that antibiotic-sensitive, sporadic isolates of \(S. typhi\) show considerable genetic diversity and probably belong to different, unrelated clones that coexist...
The high degree of genetic homology among these MDR profiles observed among the Indian MDR strains was similar to those strains, which represented 67% of all isolates studied. This observation is not surprising, given the fact that the majority of the MDR isolates belonged to a single phage type (E1). Similar findings were reported recently by Shanahan et al with MDR S. typhi isolated in India. The absolute genetic homology of the MDR strains, in contrast to the great diversity among sensitive isolates, is nevertheless remarkable, considering the fact that they were isolated from random, sporadic, and unrelated cases of typhoid fever occurring over a 12-month period. This observation may indicate that a certain molecular type or makeup may more readily acquire the resistance plasmid. The findings in this study are consistent with data previously obtained from the Indian subcontinent, which indicate that the circulating MDR strains were mostly phage type E1 and contained an antibiotic-resistance plasmid of approximately 120 MDa. Interestingly, the limited PFGE profile observed among the Indian MDR strains was similar to that noted in Pakistani strains in the present study. The high degree of genetic homology among these MDR strains was also observed in a study from Bangladesh, where these strains, which represented 67% of all isolates studied, were analyzed by phage typing, ribotyping, IS200 fingerprinting, and PCR fingerprinting. However, the Bangadesh MDR strains were not analyzed by PFGE, therefore, it was not possible to determine if the same clone was present in Bangladesh, India, and Pakistan.

A previous study from India had suggested the concurrent prevalence of sensitive and MDR strains, and the temporal behavior of these strains suggested that they were epidemiologically independent pathogens and were not in competition. By analyzing sensitive and MDR strains obtained at the same time from sporadic cases of typhoid fever, the present study has provided evidence, at the molecular level, that the two varieties are, indeed, genetically independent clones as defined by the criteria of Tenover et al. It has further been suggested that the factors involved in the transmission or establishment of the organism were not identical for these two varieties of S. typhi. The independence of these two entities of S. typhi is further suggested by the observation that the incidence of multidrug resistance has declined in recent years following the discontinuation of chloramphenicol as the drug of choice in the treatment of typhoid (Bhutta ZA 1990) years. J Clin Microbial 1996; 34:1701-1707.

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