Original Report

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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With regard to emerging infectious diseases, typhoid fever caused by multidrug-resistant (MDR) strains of *Salmonella typhi* presents a serious problem in many developing countries of the Third World.[1,2] Multidrug-resistant strains of *S. typhi* resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, with additional resistance to streptomycin, sulfonamides, and tetracyclines began to appear in the late 1980s in the South Asian region and have now spread widely to the Middle East, Africa, and Asia.[1-4] The problem is particularly acute on the Indian subcontinent, where approximately 60 to 65% of strains isolated are MDR strains. In addition, the disease associated with MDR strains tends to be more severe, with patients registering a higher morbidity score and a higher mortality rate, often with unusual complications.[3,5,6] To design rational strategies to control dissemination of such MDR strains, and also to better understand their biologic characteristics and epidemiology, the analysis of such strains, using a variety of recently available molecular methods, with higher discriminatory power compared to classic techniques, would seem to be a high priority. Molecular analysis of *S. typhi* from different parts of the world has been performed in recent years by several techniques, such as ribotyping, IS*200* typing, polymerase chain reaction (PCR)-based methods, and pulsed-field gel electrophoresis (PFGE).[7-11] This article presents the molecular analysis of antibiotic-sensitive and MDR strains of *S. typhi* from Karachi, Pakistan, using PFGE, with a particular emphasis on the coexistence of these strains in a typhoid-endemic region.

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

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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.[5] 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.[12] Genomic DNA was prepared by the method previously described.[10] Isolates were analyzed by PFGE following digestion of chromosomal DNA with the restriction endonuclease *Xba*I (5′-TCTAGA-3′), according to previously published procedures.[10] 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:

$$F = 2n_{xy}/(n_x + n_y),$$

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, complete dissimilarity.[10]

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 J3, (10%). Analysis by PFGE of these *S. typhi* isolates following digestion of chromosomal DNA with *Xba*I 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 *Xba*I 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 = 0.58–1.00). 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 = 0.98–1.00). 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,

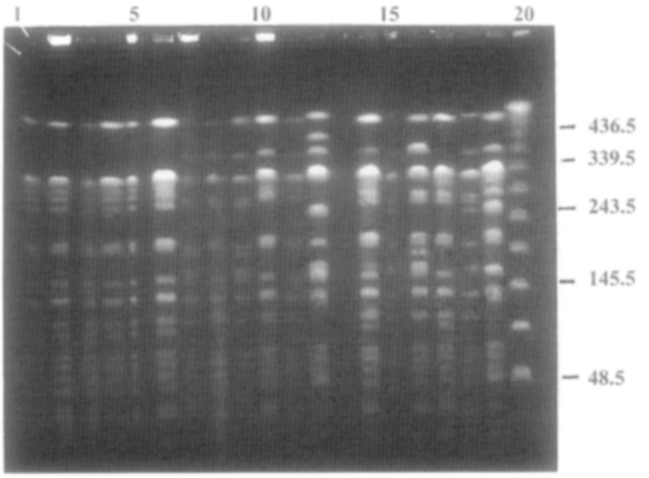

Figure 1. Chromosomal DNA PFGE profiles from antibiotic-sensitive and MDR *S. typhi* following digestion with *Xba*I (5′-TCTAGA-3′). Lanes 1–3, MDR *S. typhi* from individual patients; lanes 4–6, antibiotic-sensitive *S. typhi* isolates from individual patients with similar pattern to MDR *S. typhi* in lanes 1–3; lanes 7–19, antibiotic-sensitive *S. typhi* isolates from individual patients (lane 13 is an empty lane); lane 20, molecular weight marker (lambda PFGE marker). Kb-values indicate size and position of marker band.

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

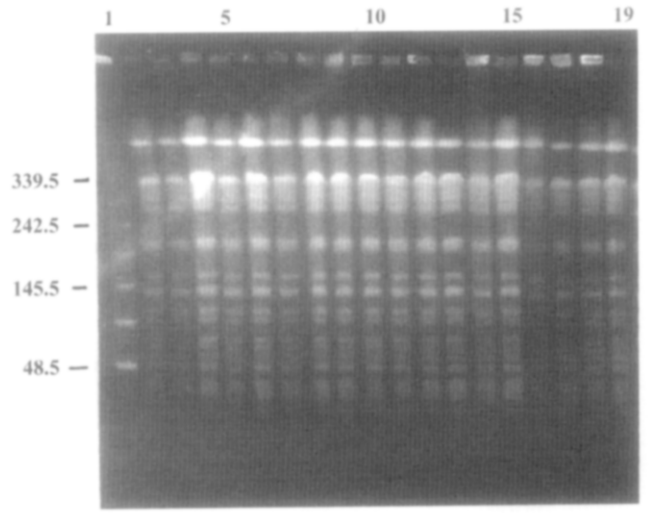

Figure 2. Chromosomal DNA PFGE profiles from MDR *S. typhi* following digestion with *Xba*I (5′-TCTAGA-3′). Lane 1, molecular weight marker (lambda PFGE marker); lanes 2–19, MDR *S. typhi* isolates from individual patients. Kb-values indicate size and position of marker band.

simultaneously, as defined by the criteria of Tenover et al.[10,13] In contrast, the MDR strains resemble isolates obtained during outbreaks of typhoid fever,[11] which are more limited in diversity and belong to a single phage type or a few closely related clones. This observation is perhaps 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.[14] 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,[1,15,16] 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 also was observed in a study from Bangladesh, where these strains, which represented 67% of all isolates studied, were analyzed by phage typing, ribotyping, IS*200* fingerprinting, and PCR fingerprinting.[16] However, the Bangladesh 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.[17] 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.[13] 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*.[17] 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. Personal communication). This would suggest that the existence and persistence of this MDR clone is independently maintained as a result of positive selection by the antibiotic.

The present study has also provided further support for the previous observation that PFGE profiles may correlate with disease severity, for example, the ability to cause fatal typhoid fever.[11] It is well established that children infected with MDR *S. typhi* are generally sicker at presentation, are more "toxic" in appearance, and have a higher incidence of disseminated intravascular coagulation and hepatomegaly, compared to those infected with antibiotic-sensitive strains.[2] The mortality rate associated with MDR strains was 4.2% as opposed to 1.4% among those infected with sensitive strains.[5,6] Unusual complications also have been reported in typhoid outbreaks caused by MDR *S. typhi*.[3] In this study it was shown that the MDR strains of *S. typhi* had a distinct PFGE profile that was present in only 3 (6%) of 50 antibiotic-sensitive isolates obtained from children with generally milder disease. Thus, information pertaining to the molecular characteristics of MDR strains may be important in future studies to examine the capability of these strains to produce more severe disease, which is believed to involve other factors in addition to resistance to antibiotics. The present study reaffirms the usefulness of PFGE in molecular typing and discrimination of individual *Salmonella* isolates for the purpose of understanding the epidemiologic behavior of these organisms and as a basis for the development of rational control strategies.

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