PULSED-FIELD GEL ELECTROPHORESIS AS AN EPIDEMIOLOGIC TOOL IN THE INVESTIGATION OF LABORATORY ACQUIRED *SALMONELLA TYPHI* INFECTION

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Abstract. Strains of *Salmonella typhi* implicated in two separate cases of laboratory acquired infection from patients and the medical laboratory technologists who processed the patients' samples were analysed by pulsed-field gel electrophoresis. Although all four isolates were of bacteriophage type E1, PFGE was able to demonstrate that the strains responsible for the two laboratory acquired cases were not genetically related. The PFGE patterns of the isolates from the MLTs were found to be identical to those of the corresponding patients after digestion with restriction enzyme AvrII. This provided genetic as well as epidemiological evidence for the source of the laboratory acquired infections.

INTRODUCTION

Laboratory acquired infections caused by bacterial, fungal, viral, rickettsial and parasitic agents have been well documented (Pike, 1979). Very frequently, the infection is picked up through routine search for the agents, epidemiological typing of strains or introduced as unknown organisms as part of a microbiology proficiency testing program.

Typhoid is endemic in Malaysia and exposure to *Salmonella typhi*, the causative organism in the laboratory is an unavoidable occupational hazard. In 1992, two cases of laboratory acquired *S. typhi* infection in two separate hospitals in Malaysia were confirmed. Bacteriophage type and the antibiotic susceptibility patterns of the strains isolated from the blood samples of the medical laboratory technologists (MLT) were similar to those of the patients' isolates handled by the MLTs. There was no other obvious source of infection and the interval between exposure to the organisms and the onset of illness were compatible with the incubation period for typhoid fever.

Bacteriophage typing has been widely used for a long time to demonstrate epidemiological associations among strains of *S. typhi* but its usefulness can be limited as a few phage types represent majority of the strains isolated here. Recently, molecular techniques such as ribotyping (Altwegg *et al*, 1989), envelope protein profiles (Franco *et al*, 1992) or the analysis of chromosomal DNA restriction patterns by pulsed-field gel electrophoresis (PFGE) (Thong *et al*, 1994) have been applied as supplementary tools in the epidemiological investigation of typhoid fever.

In this study, we use PFGE to compare the chromosomal DNA restriction patterns of the 4 strains of *S. typhi* isolated from the blood samples of the 2 patients and the respective MLTs who processed the patients' samples. Isolates were identified biochemically and confirmed by slide agglutination with specific antisera. The 4 strains were bacteriophage type E1 by the Vi-Phage typing method recommended by the International Reference Laboratory for enteric phage typing, Colindale, England. All 4 strains also had the same antibiogram:- they were resistant to ampicillin, chloramphenicol, cotrimoxazole, streptomycin and tetracycline but susceptible to kanamycin, ciprofloxacin, ceftriaxone and norfloxacin by the disc diffusion method of NCCLS (1984). The strains were maintained on nutrient agar slope soon after isolation until use.

Preparation of DNA for PFGE analysis were done as described previously (Thong *et al*, 1994). Briefly, bacterial cells were grown with shaking at 37°C in Luria broth (GIBCO, BRL) to an optical density of 0.4 at 650nm. Cells were washed 3 times with 10mM Tris-HCl/1M NaCl at pH 7.5. The bacterial cells were then embedded in 1.5 % low melt preparative grade agarose (Bio-Rad Laboratories, Calif). Agarose blocks were incubated with lysozyme solution overnight at 37°C followed by deproteinization at 50°C for 48 hours. The agarose blocks were then washed 5 times at two-hourly interval. *S. typhi* chromosomal DNA was digested

with restriction endonucleases XbaI (5'-TCTAGA-3'), SpeI (5'-ACTAGT-3') and AvrII (5'-CCTAGG-3') (New England Biolabs, USA) and DNA fragments were separated by a contour clamped homogenous electric field gel electrophoresis method on CHEF DR-II System (Bio-Rad Laboratories, Calif) in gels of 1% agarose (GIBCO, BRL) for 24 hours at 200V at 14°C with ramped pulse time varying from 1-50s. The gels were stained with ethidium bromide and photographed with a UV transilluminator (Spectroline, 302nm). The DNA size standards used were a mid-range PFG marker II (24-291 kbp) (New England Biolabs) and a bacteriophage lamda ladder consisting of concatemers with increments of 48.5 kbp (Bio-Rad Laboratories, Calif). The test strains were run twice on separate occassions to ensure reproducibility.

For each strain digested with various restriction endonucleases, 16-18 visible bands were obtained (Fig 1). The restriction endonuclease analysis (REA) patterns generated from the patients' isolates and those from the MLTs were assessed and compared visually. Isolates were considered to be genetically identical if there was complete concordance of the DNA fragment profiles and were considered different if there was a difference of one or more DNA bands.

DNA fingerprinting of the 4 isolates by PFGE after digestion with XbaI and SpeI were indistinguishable (Fig 1A, 1B) but it revealed two separate patterns with AvrII (Fig 1C), hence detecting two distinct strains responsible for the two laboratory acquired cases. The REA patterns of the strains recovered from the patients were similar to the isolates recovered from the corresponding MLTs (Fig 1).

Although the *S. typhi* isolates from both patients were of bacteriophage type E1, PFGE was able to discriminate the 2 strains as genetically unrelated and at the same time provided the laboratory evidence that the isolates recovered from the MLTs were genetically similar with that of the corresponding patients. Thus far, PFGE has demonstrated greater sensitivity and reliability in strain differentiation of *S. typhi* as this method scans for genetic variations over the entire bacterial chromosome. It can be used to complement phage typing

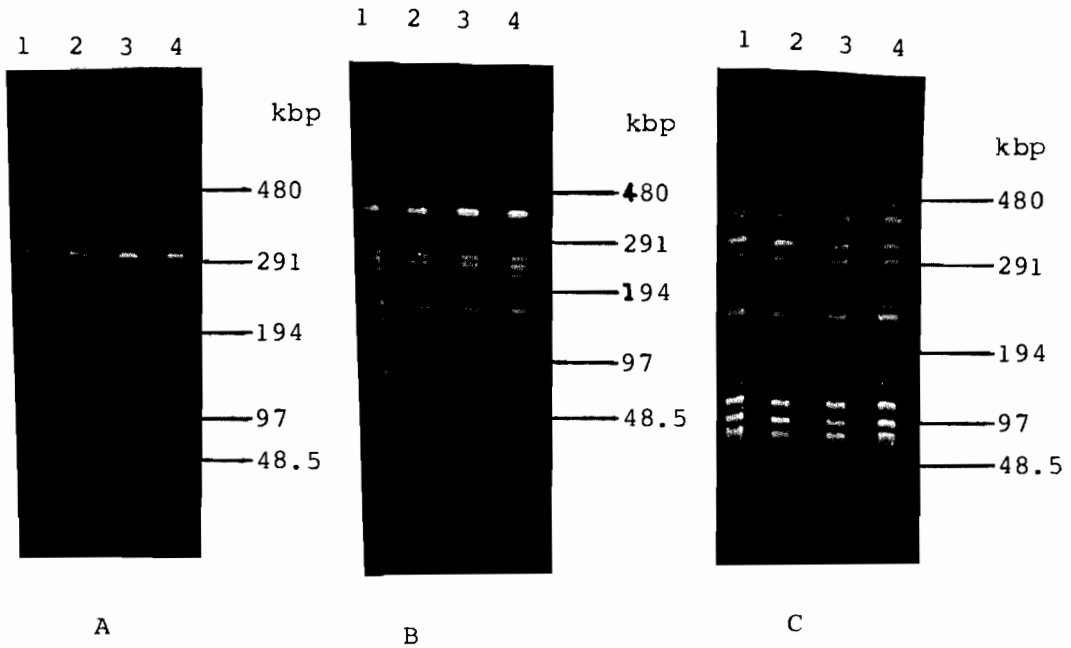

Fig 1–PFGE patterns of *S. typhi* isolates after digestion of chromosomal DNA with XbaI (A), SpeI (B) and AvrII (C). Lane 1 (patient isolate) and 2 (MLT isolate) were associated with the first case of laboratory acquired infection and lane 3 (patient isolate) and 4 (MLT isolate) with the second case.

and other genotypic techniques for effective epidemiological surveillance.

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