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RESEARCH NOTE

Rapid pulsed-field gel electrophoresis method for *Streptococcus* spp.

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Abstract. Pulsed field gel electrophoresis (PFGE) despite being a highly reproducible and discriminatory method for molecular subtyping of many bacterial pathogens has been criticised for being time-consuming. We have developed a modified, rapid PFGE method for Gram-positive bacteria, in particular, *Streptococccus* spp. Our method can be completed in only 3 days' time as compared to the standard procedure which required up to 6 days. We successfully applied this method on clinical isolates of *Streptococcus pneumoniae* (50) and Group B *Streptococcus* (50) obtained from the University of Malaya Medical Center with comparable results. The method for DNA preparation was reproducible when repeated analysis was carried out. The rapid method has the ability to process a large number of isolates in less time than the standard method. This will definitely enhance the rapid and accurate analysis of outbreaks of nosocomial or community-acquired *Streptococcal* infections.

Key Words: Rapid protocol, PFGE, *Streptococcus* spp.

Pulsed-field gel electrophoresis (PFGE) has been shown to be highly reproducible and discriminatory for the molecular subtyping of a broad range of bacteria (Swaminathan *et al.*, 2001). In the case of *Streptococcus pneumoniae*, PFGE has been successfully applied as an epidemiological tool to investigate the spread of drug-resistant pneumococci (Hall, 1998). In addition, the increase in nosocomial infection due to Gram positive bacteria is commonly reported. Lefevre and co-workers (1993) evaluated PFGE of pneumococci and found that the technique was excellent in differentiating between epidemiologically unrelated pneumococcal strains. PFGE of chromosomal DNA from drug resistant pneumococci isolated in Iceland, Portugal and France has demonstrated the clonal spread of Spanish multiresistant strains to these countries (Soares *et al.*, 1993; Gasc *et al.*, 1995; Vaz Pato *et al.*, 1995). The emergence of drug-resistant strains makes antibiotic sensitivity tests less useful in differentiation individual strains. Hence, there is a need for a discriminative, rapid and reproducible method to detect minor differences among drug-resistant strains so as to trace the source of infections. However, the main disadvantage of PFGE method is that it is time-consuming. The technique involves extracting the whole genomic DNA of a bacterial cell that is embedded in agarose. The DNA-agarose plug is then digested with a rare-cutting restriction enzyme. The large-DNA fragments are then separated by applying a pulsed electric field in a standard PFGE apparatus. The standard procedure might take up to 6 days to complete, from the day of pure culture bacterial isolation (Dipersio *et al.*, 1996). This has become a disadvantage as a clinical laboratory needs quick result especially in cases of outbreaks where a large number of isolates will have to be analysed. Thus, a PFGE method, that is simple and rapid to perform but at the same time retain the quality of the result, needs to be developed. Here, we present a modified, rapid PFGE method for the analysis of clinical isolates of *Streptococcus pneumoniae* and Group B *Streptococcus* obtained from the University of Malaya Medical Centre. The modified method is even shorter and comparable to the earlier report for rapid PFGE protocol for Gram-negative bacteria (Thong and Pang, 1996).

The PFGE method described was adapted from previously published method (Dipersio *et al.*, 1996;

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Thong and Pang, 1996). The modified, shortened protocol is as follows:

Day 1: Cells grown overnight on blood agar plates in CO_2 at 37°C were scrapped and directly transferred to 1 ml of SB buffer (10 mM Tris-HCl, pH7.5, 1 M NaCl). The cells were washed twice by centrifugation at 8000 x*g* at 4°C for 5 min. The washed cells were then resuspended in 100 µl SB and mixed with an equal volume of 1.5% low melt agarose (SeaPlaque, FMC Bioproducts, Rockland, Me) and then immediately transferred to a mold and allowed to set for 10-20 min at 4°C. The solidified agarose-cell plugs were placed in 2 ml pre-lysis buffer (10 mM Tris-HCl, 100 mM NaCl, 0.5% 20 Cetyl Ether, 0.2% Deoxycholate, 0.5% Sarkosyl, 1 mg/ml RNaseA, 1 mg/ml lysozyme, 0.1 mg/ml lysostaphin) for 2 h at 37°C. The buffer was discarded and 2 ml fresh lysis buffer [0.5 M EDTA (pH8.0), 1% Sarkosyl] containing 20 µl proteinase K (10 mg/ml) was then added and plugs were further incubated overnight (>12 h) at 50°C.

Day 2: The buffers were discarded and the DNA-plugs were thoroughly washed three times with TE buffer [1 mM Tris-HCl (pH8), 1 mM EDTA] at room temperature with gentle agitation. A slice of the DNA plug (2x3x3 mm) was digested with 10-20 Units of *Sma*I for 2-3 h at room temperature. The digested chromosomal DNA was then separated by PFGE using CHEF-DRII apparatus at 200V or 6V/cm at a temperature of 12°C in 0.5X TBE. For *Streptococcus pnemoniae*, the pulse times was ramped from 1s to 35 s for 23 h while the ramped pulsed times for Group B Streptococcus was 1s to 40 s for 24 h. Different pulse times were used for each of these *Streptococcus* spp. for optimal separation.

Day 3. After an overnight electrophoretic run, the gels were stained with 0.5 mg/ml ethidium bromide, destained in distilled water and then photographed with UV transilluminator. The DNA fingerprints were analysed visually.

We initially applied this modified rapid protocol on 50 clinical isolates of *Streptococcus pneumoniae* in our laboratory. This method was later repeated for the analysis of 50 isolates of Group B *Streptococcus* by another individual from another laboratory. For both *Streptococcus* spp., the genomic DNA obtained was of high quality as indicated by the absence of DNA degradation in an undigested agarose plug (data not shown). The DNA could be easily restricted and the digested DNA fragments were clearly differentiated by PFGE. The PFGE profiles from selected *Streptococcus pneumonia* showed that the 8-10 DNA fragments were distinctly separated (Figure 1). For Group B *Streptococcus*, the method also gave good reproducible patterns with 8-10 bands (Figure 2). The choice of the infrequent cutter that is *Sma*I was suitable for *Streptococcus* spp. analysis as the number of DNA fragments generated ranged from 8 to 10 fragments giving a defined pattern which were visually discernable. The reproducibility of the DNA banding patterns was good as identical profiles of *S. pneumoniae* were observed on repeated analysis (data not shown). The same observation was true for repeated analysis of Group B *Streptococcus*. The DNA preparation method is universal and widely applicable for other bacteria. However, the precise separation of the restricted DNA by PFGE is dependent on many factors such as the type of bacteria, choice of restriction endonucleases, pulse times, gel concentrations and the duration of the electrophoresis.

This method was modified from the Dipersio *et al.* (1996) which described the PFGE analysis for group A *Streptococcus*. The main modifications were the growth on solid medium (instead of an overnight cell culture- a saving of at least 12 h) and the use of less concentration of reagents and enzymes. The extraction of DNA from a gram-positive organism requires a harsh regimen of reagents (eg. lyzozyme, lysostaphin and mutanolysin) due to the thick cell wall constituents. In the Dipersio's method, the bacterial cells were incorporated in a plug together with the combination of lysostaphin/lysozyme and mutanolysin. During the lysis step, a combination of lysostaphin/lysozyme was added again in the pre-lysis solutions (Dipersio, 1996). In our method, lysozyme and lysostaphin were added only once in the pre-lysis step. We have shortened the pre-lysis step to 2-3 h instead of 12 h. The lysis step with proteinase K was carried out at 55°C for 12 h instead of 48 h as described in the original protocol. Following the treatment with proteinase K, the plugs were directly washed several times and digested with only 10-20 U of *Sma*I. For some pneumococcal isolates, Lefevre and co-workers (1993) had used up to 250U of *Sma*I. The reduction in the incubation period of the lysis step did not in any way affect the quality of the DNA obtained. This rapid technique may allow an analysis of a large number of strains to be done at once at a faster rate. We hope that this modified PFGE protocol will be useful to the rapid investigation on the genetic relatedness of pneumococcal isolates. In Malaysia, the increasing incidence of multidrug resistant *S.*

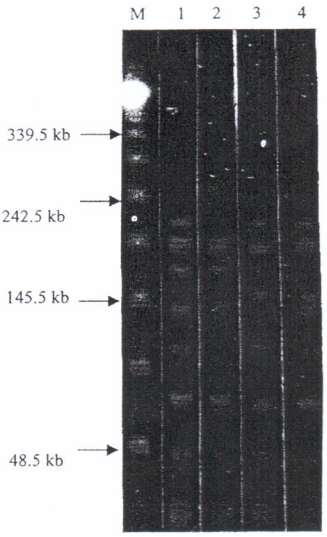

Figure 1. PFGE of representative *SmaI* digested Chromosomal DNA of *Streptococcus pneumoniae*. M= Lambda DNA concatamer standard marker. Lanes 2-4: PFGE profiles of individual isolates.

pneumoniae is also of concern. Thus the development of such a rapid method will enable faster output of accurate data to trace the source of infections.

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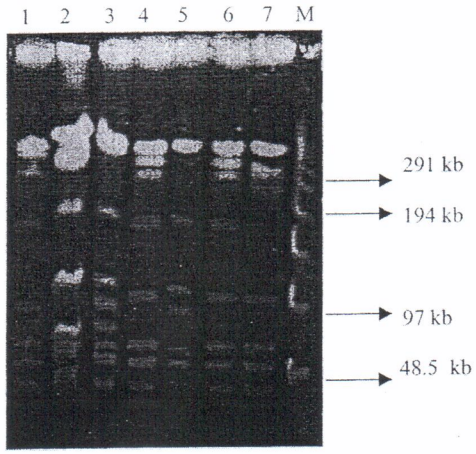

Figure 2. PFGE of representative SmaI digested chromosomal DNA of Group B *Streptococcus*. Lanes 1-7: PFGE profiles of individual isolates. M= Lambda DNA concatemer Standard marker.

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