

DNA Fingerprinting of *Mycobacterium tuberculosis* Isolates from Bangladesh by Polymerase Chain Reaction

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Abstract

Culture of *Mycobacterium tuberculosis* provides no information on the identity of a strain or the distribution of such strain in the community. In this study seventy-eight strains from Bangladesh were fingerprinted by a rapid polymerase chain reaction (PCR) in order to determine the correlation with rifampicin resistance. Purified chromosomal DNA of cultured clinical samples of *M. tuberculosis* were subjected to PCR using primers DKU 53 (CGGACTCACCGGGGCGGTTC) and DKU 55 (CCTTTCCAAGAACTGGAGTC). The PCR products, visualized after agarose gel electrophoresis and ethidium bromide staining, demonstrated that different strains of *M. tuberculosis* gave different banding patterns. Among the 78 samples PCR banding patterns of 31 strains were unique. The banding pattern of the other 47 isolates were identical to at least another isolate. The relationship between two samples from the same house could be established but the relationship between other samples with identical pattern could not be established. Association of specific PCR patterns with rifampicin resistance was not found indicating that no special rifampicin resistant strain was spreading in Bangladesh. (*J Infect Dis Antimicrob Agents* 1997;14:1-3.)

INTRODUCTION

Tuberculosis is still a world health problem. About one billion people are infected by *Mycobacterium tuberculosis* throughout the world. The morbidity and mortality are about 10-20 and 3 million per year respectively and only 1/3 to 2/3 of the total cases are being diagnosed (1). The human immunodeficiency virus (HIV) is having profound impact on the tuberculosis problem and both developed and developing countries (2). Tuberculosis affects at least 6 million Asians every year and half of these are infectious. The actual number of new cases in Asia is 3.7 times greater than that in Africa and the annual risk of infection in Southeast Asia region is 2 percent (3). Tuberculosis is also one of the important causes of morbidity and mortality in Bangladesh where drug resistance was also commonly

found. Since testing the drug resistance is time-consuming, we evaluated a rapid PCR method (4) for strain characterization of *M. tuberculosis* isolated from patients in Bangladesh in an attempt to correlate the pattern with drug sensitivity in particularly rifampicin.

MATERIALS AND METHODS

Bacterial strains and DNA preparation

Seventy-eight samples from Bangladesh were studied. *M. tuberculosis* were cultivated in Lowenstein-Jensen media for 4-6 weeks. Isolation of chromosomal DNA was done by an enzymatic method (5). Briefly the cells were transferred to a microcentrifuge tube containing 400 μ l of TE (10 mM Tris HCl pH 8.0 and 1 mM EDTA) buffer and killed by heat at 80°C for 20 min. 50 μ l of 10 mg/ml lysozyme solution was added. After

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incubated overnight, 75 μ l of 10 percent SDS/proteinase K mixture were added to the cells suspension which was then incubated at 65°C for 10 min. 100 μ l of 5M NaCl and 100 μ l of CTAB/NaCl solution were sequentially added. The suspension was vortexed until the liquid content become white and then was incubated for another 10 min at 65°C before extracted once with 750 μ l chloroform/isoamyl alcohol. The aqueous phase was then precipitated with 0.6 volume of 2-propanol. After washed with 70 percent ethanol the pellet was redissolved in 200 μ l of TE buffer pH 8.0. The concentration of DNA was determined by spectrophotometric absorbance at 260 nm.

PCR Condition

Amplification was carried out in 50 μ l volume containing 1.5 μ M of each primer (DKU53:CGGACT CACCGGGCGGTTC and DKU55:CCTTTCCAAG AACTGGAGTC), 5 unit of *Taq* polymerase and 100 ng of DNA. The mixture was overlaid with 50 μ l of mineral oil. PCR was performed in a thermocycler, with each cycle including 1 min denaturation at 94°C, 1 min annealing at 50°C, 1 minute extension at 72°C.

A total of 30 cycle was performed.

RESULTS

Among the 78 patients, 55 were male (70.5%). The most common age group was 25-44 years. All of the patients were HIV negative. The patients were positive by direct microscopy by Zeihl-Neelsen's staining. Drug sensitivity testing revealed that 13 isolates were resistant to rifampicin and 2 isolates to isoniazid. Both isoniazid-resistant isolates were also resistant to rifampicin. No isolates were found resistant to streptomycin or ethambutol.

PCR amplification with DKU53 and DKU55 of the strains produced bands of various intensity consisting of four to six major bands, and a number of weaker bands also. The combination of these two primers produced several different polymorphic bands (Fig.1). Some polymorphic bands appeared faint and were not very reproducible. But distinct bands are reproducible and are useful for strain characterization. The pattern of 31 samples were unique while 47 isolates showed PCR pattern identical to at least another isolate. Five of

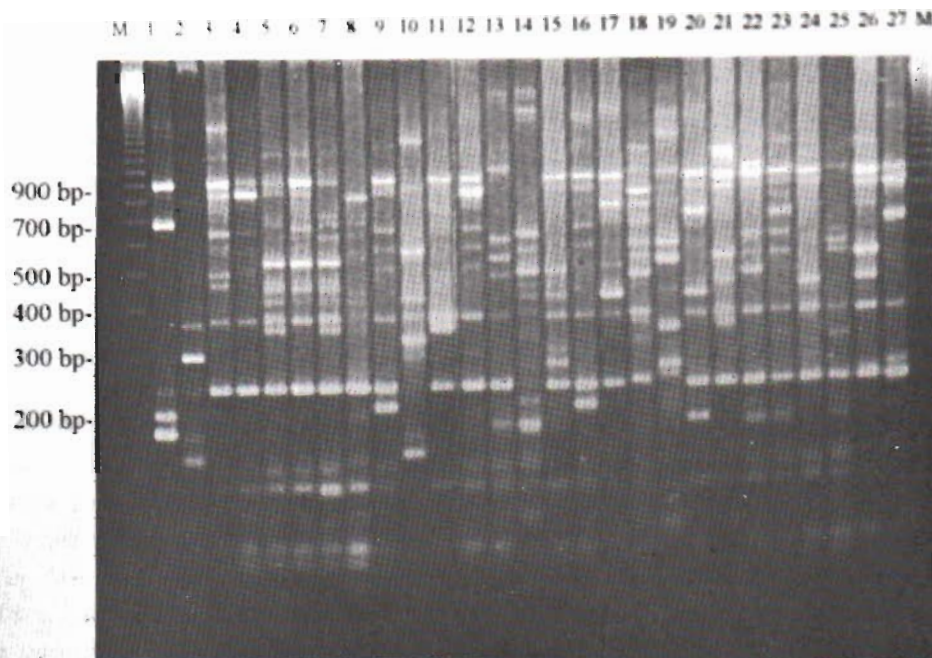


Fig. 1 The PCR products of some of the *M. tuberculosis* isolates from Bangladesh. Lane 9 and 17 showing the identical fingerprinting, they are cousin residing in the same apartment. The relationship between other isolates with identical fingerprint was not known. The patients from 9 to 28 were from the same center. Lane 4,6,8,9,11, were the strains which were resistant to rifampicin. Lane 8 and 11 were the strains which are resistant to both the drug isoniazid and rifampicin.

the rifampicin-resistant isolates had unique fingerprinting pattern while the other 8 rifampicin-resistant isolates had the pattern identical to at least one another isolates. Of these latter group, 4 isolates had fingerprints identical to rifampin sensitive isolates while the other 4 could be divided into two groups of identical pattern. The fingerprints of both multidrug-resistant isolates were different from each other.

DISCUSSION

Analysis of the samples from Bangladesh showed that male patients are more (70.5%) than the female (29.5%). The reason was due to socio-cultural condition of the country where male were more exposed to the diseases. Moreover females might also be reluctant to come to the clinics. The disease was more in the age group of 25-44 year. This age group was the active population group of the country and were more exposed group. Similar distribution was also reported in Singapore and Philippine (3).

The use of PCR for differentiating the strains of *M. tuberculosis* is a rapid and simple way of typing bacterial isolates for epidemiological purposes. By the use of primers DKU53 and DKU55 polymorphism could be obtained suggesting that this method would be a method of choice when rapid epidemiological investigation is needed. Association of specific PCR patterns with rifampicin resistance was not found. This suggested that no special rifampicin resistant strain was spreading in Bangladesh. Rifampicin resistance were found to occur due to the point mutation in β subunit of RNA polymerase gene. Thus a resistant isolate can occur easily from a sensitive isolate when the patient do not comply to the treatment regimen.

In conclusion the rapid PCR method is of value for strain characterization of *M. tuberculosis* in Bangladesh, although it cannot be used for identifying rifampicin resistance which probably needs more sophisticated technique such as PCR-SSCP (6) or heteroduplex formation analysis (7).

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