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Prevalence of *Mycobacterium tuberculosis* Beijing genotype and its association with drug resistance in North India

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Abstract The global presence and rapid dissemination of Beijing genotype of *Mycobacterium tuberculosis*, makes it an important issue of public health. Its presence and association with multi-drug resistance has been shown in many settings. In present study we tried to find its prevalence and association with drug resistance in North India. One hundred and twenty four *M. tuberculosis* isolates were analyzed with spoligotyping, further drug susceptibility testing was done by 1% proportional method. Out of these, 11 (8.9%) *M. tuberculosis* isolates were identified as Beijing and 113 (91.1%) as non-Beijing genotypes. While looking at their drug susceptibility patterns, 6 (54.5%) & 22 (19.5%) were found to be multi drug resistant

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(MDR) among Beijing and non-Beijing isolates respectively. Our study concluded that the Beijing strains were not so common in north India and these strains do not fully associate with MDR.

Introduction

Beijing family genotypes of *M. tuberculosis* (MTB) were first recognized in 1995 and are accounted for 86% of the tuberculosis isolates from Beijing, China, and a high proportion of isolates from Mongolia, South Korea, Hong Kong, Malaysia and Vietnam. In other regions like Finland and India, the presence of Beijing family genotype is relatively rare [1]. The *M. tuberculosis* belonging to Beijing family has increased ability to spread, cause the diseases and has higher association with drug resistance in comparison to non-Beijing MTB isolates [2]. In previous studies from Germany, Cuba, Estonia, Russia, and South Africa it was documented that the transmission of drug resistance have association with Beijing family genotype strains [3]. Strain W which is a highly drug resistant strain; reported from the United States also belongs to the Beijing family [3].

Outbreaks of MDR tuberculosis due to Beijing genotype have been reported in several parts of the world [4]. Researchers are concerned that the Beijing genotype may have a predilection for developing drug resistance and may be spreading worldwide, perhaps as a result of increased virulence [3,5]. Thus, a better understanding of the clinical and epidemiological relevance of *M. tuberculosis* Beijing/W lineage may allow the development of better strategies for TB control.

In the present study, we have attempted to assess the prevalence of *M. tuberculosis* Beijing genotype and its drug resistance pattern in North India.

Materials and methods

Strains

One hundred and twenty four randomly selected *M. tuberculosis* isolates from three different locations of North India were included, among these, 83 from eastern Uttar Pradesh (SA-1), 26 from Sawai Madhopur, Rajasthan (SA-2) and 15 isolates from Buxar, Bihar (SA-3). For the isolation of these MTB isolates, the specimen were collected during Oct. 2004-Dec. 2007 for SA-1 and for SA-2 and SA-3 the duration was Oct. 2004 to March, 2005. The specimens were processed by modified Petroff's method and inoculated on Lowenstein Jensen (LJ) media in duplicate. After further incubation the growth was observed and the MTB isolates were characterized using standard methods like; niacin test, growth on PNB containing media and catalase test [6] at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

DNA isolation

DNA was isolated as previously described [7]. In this method the DNA was extracted by phenol and chloroform. After extraction the DNA was precipitated by 70% ethanol and kept in TE (Tris EDTA buffer, pH 8.0).

Spoligotyping

Spoligotyping was performed on genomic DNA to detect the Beijing genotype (1—34 spacers absent) by using the standard method [8] with the help of commercially available kit (Isogen Biosciences, BV, Maarsen, The Netherlands). In brief the DR region was amplified by previously described sets of primers. After amplification the amplified DNA was hybridized on to the membrane which contains the corresponding probe for each spacer (1—43). The hybridization pattern was visualized with a chemiluminescence system, using Enhanced Chemiluminescent detection system (Amersham Biosciences, Buckinghamshire, United Kingdom). Proper controls (H37Rv, *M. bovis* BCG and Negative control) were used with each experiment. The pattern of each isolate was compared with the international spoligotyping database SpolDB4.0 [9].