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Original Article

Identification of Leptospira using Arbitrarily Primed Polymerase Chain Reaction

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Abstract

Introduction: Leptospirosis is a zooanthroponotic disease caused by the spirochaete, *Leptospira interrogans,* sometimes leading to multiorgan failure and death in humans.

Materials and Methods: A total of 45 leptospiral isolates from patients with leptospirosis, who attended the medicine department of the hospital in Kolenchery, during the period January 2000-June 2002 were stocked till the time of the study. Arbitrarily primed PCR was carried out using the primer PB - 1: 5'- GCG CTG GCT CAG - 3'.

Results: The DNA extracts gave bands at 23 kb region. In arbitrarily primed polymerase chain reaction, for most serovars, differentiating bands were observed between 200 and 1200 bp. The predominant serovar in the region was autumnalis (38%) followed by pyrogenes (16%).

Conclusion: A database with distinctive genetic patterns of pathogenic leptospires isolated from humans as well as animals of a particular region is necessary and should be taken up by reference laboratories since new patterns may crop up due to the inherent variability of the repetitive element.

Key words: AP-PCR, genotyping, leptospira, leptospira serovars

INTRODUCTION

Leptospirosis is a zooanthroponotic disease caused by the spirochaete, *Leptospira interrogans,* sometimes leading to multiorgan failure and death in humans. Identifying and characterizing the pathogenic serovars of leptospira still remain a daunting task for most leptospirologists. Methods of identification of *L. interrogans* serovars are very complex. Arbitrarily primed polymerase chain reaction (AP-PCR) which amplifies fragments of DNA of a few kb from a genome using a single primer produces distinct banding patterns which help in genotyping. In this study, leptospira isolates from clinical cases were characterized using AP-PCR, with a primer of 12 bp. Work with the same primer was carried out earlier by other workers.[1,2]

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Objective

As genomic variations occur between serovars of different geographical regions, there is a strong need for obtaining a database of local serovars. The study was aimed to genotype leptospira isolates from these regions.

MATERIALS AND METHODS

A total of 45 leptospiral isolates from patients with leptospirosis, who attended the medicine department of the hospital in Kolenchery, during the period January 2000-June 2002 were stocked till the time of the study. Preliminary serotyping by microscopic agglutination test (MAT) was carried out using antisera obtained from the WHO reference laboratory, Amsterdam following procedures mentioned in the guidelines.[3]

Standard Reference Strains used in the Study

The standard reference strains were obtained from the WHO Collaborating Centre for Reference and Research on Leptospirosis, Brisbane, and Queensland, Australia. Andamana (strain CH 11), australis (strain Ballico),

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autumnalis (strain Akiyami), ballum (strain Mus 127), bankinang (strain Bangkinang I) canicola (strain Hond Utrecht IV), carlos (strain C3) cynopteri (strain 3522C), grippotyphosa (strain Moskva V), hebdomadis (strain Hebdomadis), icterohemorrhagiae (strain RGA), jalna (strain Jalna), javanica (strain Veldrat Bat 46), mini (strain Sari), Pomona (strain Pomona), panama (strain CZ214K), ranarum (strain ICF), ramisi (strain Musa), sejroe (strain M84), and tarassovi (strain perepelicin).

Local Reference Serovars

Eight isolates identified earlier up to the strain level at the WHO reference laboratory, Amsterdam by serological tests were used for the purpose of referencing since these were the only local field isolates identified up to the strain level at a reference laboratory (referred here as "local reference serovars").

DNA Extraction

The procedures for DNA extraction on all the strains were carried out as per the protocol mentioned by Ramadass *et al.*[2]

AP-PCR

Primer PB-1, 5'-GCG CTG GCT CAG-3' was used for AP-PCR analysis of all the leptospira stains isolated as well as for reference serovars. Each 50µl of PCR mixture contained 2 µl of purified DNA extract, 0.3 µM primer, 250 µM of each dNTP, 3 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase in 10 mM Tris. HCl (pH 9.0) and 50 mMKCl.

The rest of the procedures for AP-PCR were carried out as per the protocol mentioned in the publication.[2]

The patterns were compared with:

1. Standard reference serovars,
2. Local reference serovars, and
3. Previous publications employing AP-PCR with the same set of primer.[1,2]

RESULTS

The DNA extracts gave bands at 23 kb region. In AP-PCR, for most serovars, differentiating bands were observed between 200 and 1200 bp (Figure 1).

The incidence of various isolates is given in Chart 1. The predominant serovar in the region was autumnalis (38%) followed by pyrogenes (16%).

DISCUSSION

Only few workers[1,2] have reported the findings of AP-PCR using similar primer and conditions. Some of

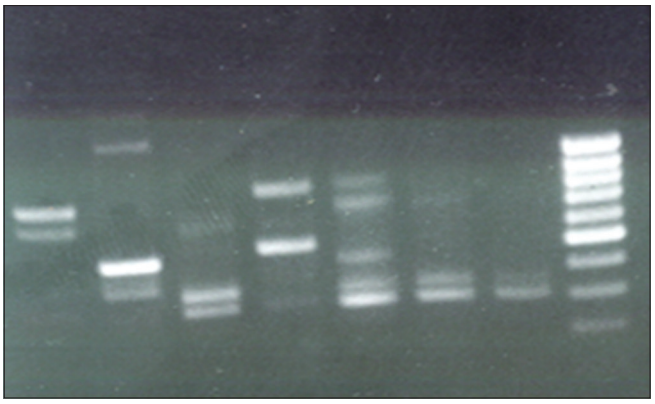

Figure 1: Patterns obtained using arbitrarily primed polymerase chain reaction of a few isolates. Standard reference serovars: Lanes: 1 - ballum, 2 - pomona, 3 - icterohemorrhagiae, 4 - CH 11. Isolates: Lanes: 5 - pyrogenes (local reference serovar), 6 - autumnalis, 7 - autumnalis, 8 - DNA marker

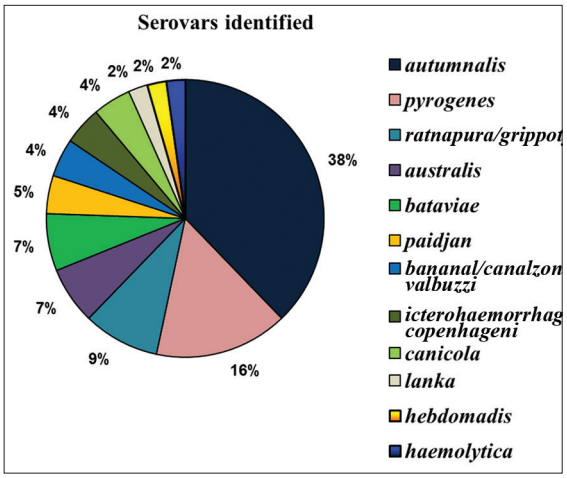

Chart 1: Serovars identified by arbitrarily primed polymerase chain reaction

the isolates gave patterns similar to those published earlier.[1,2] However, patterns of icterohemorrhagiae, Lanka, ratnapura/grippotyphosa did not match with any of the reference strains used in the study. Minor differences of one or more bands were observed in the case of some serovars of autumnalis. Two of the local reference serovars identified as serovar autumnalis strain Akiyami A at WHO Reference Centre, Amsterdam gave slightly varying patterns and were more in concurrence with the autumnalis isolates used in the study than with reference serovars. The patterns were more consistent with those of the local reference serovars indicating a strong need for in-laboratory standardization for identification purposes. Some patterns were difficult to identify. A good backup of the preliminary serogrouping with MAT helped to a great extent.

The results of the study showed that though PCR-based fingerprinting does facilitate characterization of pathogenic

leptospires unless the prevailing endemic serovars are primarily genotyped by AP-PCR, genomic characterization using this method becomes a very difficult task. Sometimes the bands are not poignant enough to visualize clearly and the comparison becomes difficult as minor differences may interfere with the identification. Variations may exist between reference strains and clinical or field specimens of the same serovar if they occupy different ecological niches.[4] Moreover, profiles may be affected by the primer used, the quantity, and quality of the DNA template and the electrophoresis conditions and has to be applied with caution.[5]

Hence, it is not a stand-alone test and being technically demanding cannot be carried out routinely in most laboratories for identification purpose as it is difficult to standardize or generate data that can be exchanged digitally.[6,7] There are reports of better discriminatory powers in typing leptospires using primers like M16[8] compared to primers like PB-1 used in this study. Until better DNA-based identification tests are developed and validated, it will be better for clinical laboratories to retain the serological classification of pathogenic leptospires which though labor-intensive is easier for basic serogrouping.

CONCLUSION

A database with distinctive genetic patterns of pathogenic leptospires isolated from humans as well as animals of a particular region is necessary and should be taken up by reference laboratories since new patterns may crop up due to the inherent variability of the repetitive element.

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