Short Communication

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Antigenic and genetic relatedness of *Leptospira* strains isolated from the Andaman Islands in 1929 and 2001

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Leptospirosis is a major public health problem in Andaman Islands. Several strains of *Leptospira* have been isolated from the Andamans over the years. Leptospire isolates recently from human cases were compared with one of the earliest available isolates from these islands, dating back to 1929, to study their serological and genetic relatedness. Randomly amplified polymorphic DNA (RAPD) fingerprints of the isolates, generated with a primer used previously to differentiate between *Leptospira* species and serovars, revealed that some of the recent isolates were genetically identical to the 1929 isolate. The antigenic properties of these strains, as revealed by microscopic agglutination tests with group-specific rabbit antisera and mAbs, were also similar. These findings suggest that a *Leptospira* strain originally isolated in 1929 has possibly persisted in these islands for over 70 years and continues to cause acute leptospirosis in humans.

Introduction

Leptospirosis is emerging as one of the world's most widespread zoonotic diseases (Plank & Dean, 2000; Yang *et al.*, 2001; Levett, 2001). The earliest authentic report of leptospirosis from India dates back to 1929, when Taylor & Goyle (1931) isolated 28 strains of leptospire from 78 suspected cases showing typical signs and symptoms of Weil's syndrome among convict labourers in the Andaman Islands. No further information was available regarding the status of leptospirosis in these islands until 1988, when post-monsoon outbreaks of a febrile illness with haemorrhagic tendencies started to appear. The mortality rate among patients was very high. Despite several efforts, the aetiology could not be established and, hence, the name 'Andaman haemorrhagic fever' (AHF) was given to this clinical entity. The clinical presentation during these outbreaks was characterized by sudden onset of fever, body ache, myalgia, cough, with or without haemoptysis and other haemorrhagic tendencies. Based on serological evidence, Sehgal *et al.* (1995) confirmed the cause of these outbreaks as leptospirosis. Bacteriological confirmation was subsequently established (Sehgal *et al.*, 2000). Since then, several isolates of leptospire have been obtained and attempts have been made to characterize these strains serologically and by using molecular techniques. The present study was undertaken with the specific objective of examining any serological and genetic relatedness between the recent isolates and the isolates obtained in 1929.

Methods

Strains and isolates. Strain CH31, belonging to serogroup Grippotyphosa, was originally isolated from a patient in the Andaman Islands in 1929 (Taylor & Goyle, 1931). This strain was obtained from the WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis, Amsterdam, The Netherlands. Four isolates, designated D22, Mg47, Mg51 and Mg100, were recovered from suspected cases attending a primary health centre on South Andaman in recent years. All cultures were maintained in EMJH culture medium (Difco) at 30 °C.

Serotyping

Microscopic agglutination test (MAT) with group sera. All five isolates were screened against 36 group-specific rabbit antisera representing 23 serogroups following standard procedures (Wolff, 1954). An isolate was considered to belong to the serogroup of the group serum that gave the highest titre (Dikken & Kmety, 1978).

MAT with mAbs. A panel of four mouse mAbs (F71C3, F71C9, 165C3 and 165C8) developed at the Dutch Royal Tropical Institute (Amsterdam, The Netherlands) that distinguish serovars of serogroup Grippotyphosa was used. Microscopic agglutination was performed using each strain against all the mAbs in the panel. The antigenic profile was constructed by plotting the reciprocal titre against the mAb on a semi-logarithmic scale. This pattern for each strain was then compared with that of CH31.

Preparation of genomic DNA. Genomic DNA of the bacterial strains was extracted following the method described by Boom *et al.* (1990). DNA was dissolved in Milli-Q water and used for randomly amplified polymorphic DNA (RAPD) analysis.

RAPD analysis. The reference primer PB1 (5'-GCGCTGGCTCAG-3'), used previously to differentiate between *Leptospira* species and serovars (Brown & Levett, 1997; Ramdass *et al.*, 2002), was used to

Abbreviations: MAT, microscopic agglutination test; RAPD, randomly amplified polymorphic DNA.

generate RAPD fingerprints for each strain. PCR was performed at least twice by different technicians using a DNA Engine PTC 200 (MJ Research) in 50 µl reaction volumes with 50 ng purified DNA, 2 µM primer, 250 µM of each dNTP, 15 mM MgCl₂, 0.5 U *Taq* polymerase in 10 mM Tris/HCl (pH 9.0) and 50 mM KCl. The temperature program consisted of one cycle of 3 min at 97 °C, 1 min at 40 °C and 1 min at 72 °C, four cycles of 1 min at 97 °C, 1 min at 40 °C and 1 min at 72 °C, 24 cycles of 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C and one cycle of 1 min at 95 °C, 1 min at 55 °C and 7 min at 72 °C. Reaction products were electrophoresed on 20 cm 1% agarose gels, stained with 0.5 µg ethidium bromide ml⁻¹ (Sigma) and viewed and photographed under UV light.

Results and Discussion

MAT with 36 group-specific rabbit antisera revealed that all the isolates belonged to the serogroup Grippotyphosa. Antigenic patterns of all five isolates against the panel of four mAbs were similar (Fig. 1). Furthermore, it was found that the antigenic profiles of all the isolates were similar to the antigenic pattern of CH31, indicating a close serological relationship between isolates D22, Mg47, Mg51 and Mg100 and strain CH31 (Fig. 1).

RAPD or arbitrarily primed PCR is increasingly being used for typing of leptospiral serovars because it is both simple and rapid and is therefore often preferred over other methods like cross-absorption agglutination test and nucleic acid hybridization (Ramdass *et al.*, 2002).

The primer PB1 used in this study has the ability to discriminate between different species and serovars of leptospires (Brown & Levett, 1997; Ramdass *et al.*, 2002) and has been used successfully in the typing and identification of several leptospiral serovars. RAPD fingerprints of the 1929 isolate CH31 and the recent isolates D22, Mg47, Mg51 and Mg100 were essentially the same (Fig. 2). At least six different bands were generated with this primer in PCR and they were well spread over the size range of 2000 to 300 bp. These results suggest that the recent isolates D22, Mg47, Mg51 and Mg100 are genetically similar to the 1929 isolate.

The findings of the present study show the similarity in antigenic and genetic nature of some *Leptospira* strains isolated more than 70 years apart from the Andaman Islands. Bacteria, with their simple genetic organization and very short generation times, are more susceptible to genetic

Fig. 1. Antigenic properties of isolates with mAbs F71C3, F71C9, 165C3 and 165C8.

Fig. 2. RAPD banding patterns of the isolates amplified with primer PB1 in PCR. Lanes: 1, marker (100 bp ladder; New England Biolabs); 2, CH31; 3, D22; 4, Mg47; 5, Mg51; 6, Mg100.

change over a period of time than are most higher organisms. Our preliminary study, however, shows that a *Leptospira* strain has undergone no apparent change in its antigenic and genetic makeup over a time span of more than 70 years. This might be because genetic exchange mechanisms are lacking in leptospires (Taylor *et al.*, 1991) and these organisms are genetically more refractory (Hardham & Rosey, 2000) than most other bacteria. However, in order to lend further support to these preliminary findings, genetic comparisons

of the present isolates with the 1929 isolate, employing other tools such as restriction enzyme analysis, PFGE, nucleic acid hybridization and RFLP analysis are currently in progress.

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