Paradoxical drug sensitivity of *Leishmania donovani* Promastigotes: an *in vitro* study by Agar elution and 96 well plate methods

Leishmaniasis, a neglected disease is a growing health problem in many parts of the world, with about 350 million people living in areas of disease endemicity and about 2 million new cases each year. Agar elution technique is widely in usage to determine the drug sensitivity of slow growing bacteria like *Mycobacterium* spp., which require up to 10 days to form visible colonies in response to any particular drug. The promastigotes of *Leishmania donovani* forms colonial growth on agar-based media after incubation for about 8 days. As both *Leishmania* spp. and *Mycobacterium* spp. forming colonial growth on agar-based media in 7-14 days, we tried to apply the agar elution method, which is generally in use to determine the drug sensitivity of *Mycobacterium* spp., to determine the drug sensitivity of promastigotes of *Leishmania donovani*. The results were compared with the standard 96 well dilution plate method (broth dilution).

Sodium stibogluconate (SSG) resistant (R) and sensitive (S) isolates of *Leishmania* promastigotes were inoculated (10^7 cells/ml) on the surface of blood agar plates [10%, v/v, defibrinated rabbit blood in commercial blood agar base (Blood Agar Base No.2; HiMedia Laboratories, Mumbai, India)] by streaking. The drugs, SSG (Albert David Ltd, Kolkata) and Amphotericin-B (AmB) (Sarabhai Chemicals, Vadodara), which are commonly used for treatment of Visceral Leishmaniasis (VL) in India, were impregnated in sterile paper discs (Whatman No.1, 5mm diameter) at varying concentrations and placed on the surface of inoculated plates. In another set, the drugs were serially diluted in Locke's solution with 10 % Foetal Calf Serum in a 96 well plate and seeded with the promastigotes (2X10^5 Cells/ml) of each isolates. Agar plates (solid medium) and 96 well plates (contains liquid medium) were incubated respectively for 8 and 3 days at 25°C.

After incubation for 3 days, the promastigotes of SSG R/S isolates showed clear sensitivity pattern to both SSG and AmB, in 96 well plates. The MIC of SSG (R) isolate was 5mg SSG/ml and 0.078 µg AmB /ml and the same for the SSG (S) isolate was 0.15 mg SSG/ml and 0.078 µg AmB/ml. But the results of agar elution is unexpected and highly contradictory, pale white, dome-shaped and mucoid colonies of promastigotes evenly formed all over the plate, irrespective of discs loaded with drugs. Even the highest possible concentration of drugs that can be achieved in disc, i.e., 500µg SSG/disc and 25µg AmB/disc was not sufficient to stop the growth of promastigotes of both isolates on the solid medium (Fig.1). This experiment was run three times.

![Fig 1. Colonial growth of Sodium Stibogluconate (SSG) responsive (A) and unresponsive (B) isolates of Leishmania donovani promastigotes in the presence of discs, loaded with SSG and Amphotericin B (AmB)](image)
This $in vitro$ study revealed that, the testing drug could act appropriately if the promastigotes are suspended in liquid phase. The same drugs would not be ineffective if it is impregnated in paper disc and hence, the agar elution technique is not suitable for the determination of drug sensitivity of the promastigotes of Leishmania.

Centre for Research in Medical Entomology, (Indian Council of Medical Research),
No. 4, Sarojini street, China Chokkikulam,
Madurai 625002, India.
Email: mmuniaraj@yahoo.com

REFERENCES


ACKNOWLEDGEMENTS
I am thankful to Dr. P. Das, Director of RMRIMS, Patna, India for providing facilities to carry out this work and the assistance of Dr. P. K. Sinha, Deputy Director, RMRIMS, S.K. Thevary, N. Kumar, Dheerendra is also greatly acknowledged.

M. Muniaraj