International Journal of Trend in Innovative Research (IJTIIR)
Available Online @ www.ijtiir.com

Pathogenicity of Drug Resistant Isolates of M.Tuberculosis in Guinea Pig Animal Model

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***Abstract*--** There is very limited information available on pathogenicity of multi drug resistant M.tuberculosis strains, isolated from Indian patients in the animal model.This study was attempted to ascertain any difference among various groups of drug resistant field isolates of M. tuberculosis in Bangalore. The study revealed an interesting finding that among various groups of drug resistant isolates of M. tuberculosis, group (SRSR) that was sensitive to Streptomycin and Rifampicin but resistant to Isoniazid and Ethambutol produced severe and extensive disease in the Guinea Pig animal model compared to all other drug resistant profile groups. Further investigation are needed from such drug resistant profile in different parts of India preferably with genome sequence of each strain that may check the menace of MDR TB in India.

I. INTRODUCTION

Multi-drug-resistant tuberculosis (MDR-TB) is emerging as a major challenge to the programme-managers worldwide besides dealing with TB-HIV co-infection (Ref:3&4-Gopinath, Tubercle 89,2009). Globally, the incidence of MDR TB appears to be rising, as revealed in the WHO 2008 report (World Health Organization. Anti-tuberculosis drug resistance in the world, Report #4.2008). As per this report, China and India contributed 50% of MDR-TB burden in the world. Resistance of *M.tuberculosis* to an anti-tuberculosis drug is usually the result of spontaneous genetic event and worse, "a man made amplification of natural phenomenon". Drug resistance acquired during chemotherapy results in changes impairing the growth properties of tubercle bacilli. Mycobacterium uses various mechanisms to evade killing by drugs, including mutations in genes that code for drug target proteins (Ramaswamy S., and Musser J.M.: Molecular genetic bases of antimicrobial agent resistance in *Mycobacterium tuberculosis*: Tuber. Lung Dis.; 1998, 79, 3-29). Concomitantly with these changes infectiousness and pathogenicity of drug resistant bacilli get affected adversely, as seen in guinea pig animal model (Cohn M.L., and Devis C.L.: Infectivity and Pathogenicity of Drug-Resistant Strains of tubercle bacilli in guinea pigs: Amer. Rev. of Resp. Dis.; 1970, 102, 97-100). Most important application of the guinea pig model has been in understanding the fundamental relationship between tubercle bacilli and its mammalian host (Smith D.W, Wiegeshaus E.H. What animal models can teach us about the pathogenesis of tuberculosis in man. Rev Infect Dis 1988 & Smith D.W.: A guinea pig model of experimental airborne tuberculosis for the evaluation of the response to chemotherapy: the effect on bacilli in the initial phase of treatment, Tubercle, 1991, 72, 223-227). Many other investigators have reported about the virulence of drug-sensitive clinical isolates of *M. tuberculosis* from different parts of the world (Mitchison et al Tubercle 1960; Naganathan et al Tubercle 1986; Prabhakar et al Tubercle 1987).

Studies on drug–resistant strains of *M.tuberculosis* in animal models were earlier limited to only those drug-resistant strains that grew poorly on 7H10 oleic acid-albumin-agar or required casein hydrolysate for growth. They had shown that the loss of catalase activity in isoniazid-resistant tubercle bacilli resulted in the loss of pathogenicity (Cohn M.L., and Devis C.L.: Infectivity and Pathogenicity of Drug-Resistant Strains of tubercle bacilli in guinea pigs: Amer. Rev. of Resp. Dis.; 1970, 102, 97-100). Recent studies reporting *in vivo* virulence and pathogenicity of experimental *M.tuberculosis* infection in animals have used either laboratory grown H37Rv or Erdman *M.tuberculosis* strains (Palanisamy G.S., DuTeau N., Eisenach K.D., Cave D.M., Theus S.A., Kreiswirth B.N., Basaraba R.J., and Orme I.M. : Clinical strains of *Mycobacterium tuberculosis* display a wide range of virulence in guinea pigs: Tuberculosis 2009;89:203-209). The sequences of the loci associated with multi-dug resistance in good number of clinical isolates of *M.tuberculosis* from India have been recently reported, which may help to develop future treatment regimens & drug deign strategies, (Noman Sidiqe, Katch VM: Molecular…in north India. 2002 Anttimicrobial.. vol46,443-450). Another study from India on comparative growth pattern of 2 dug-sensitive & 2 MDR strains had shown some difference, indicating an urgent need to understand the biological differences significant for the pathogenesis, transmission dynamics, alternate modes of treatment, new vaccine development. (Ref: Sakshi…….Katoch VM, 2009 IJMR vol 130, 58-62). Keeping in view the scarcity of basic data on pathogenicity of drug-resistant clinical isolates of *M.tuberculosis* from India, which WHO has identified as a major hot-spot for TB infection, the purpose of this study was to find variations in pathogenicity, if any, among different drug-resistant profiles of *M.tuberculosis* field isolates, when tested in albino guinea pig model.

II. METHODS

A. Source of Isolates & Bacteriological Procedure:

The isolates of drug resistant/sensitive tubercle bacilli to be tested in this study have been obtained from multi drug resistant (MDR) suspects referred to NTI laboratory

for culture and drug susceptibility testing (DST) from Rajasthan and West Bengal states of India. The sputum specimen was transported in sterile containers, each containing 5 ml of sterile1% cetyl pyridinium chloride (CPC) solution. For culture and DST standard procedures, as per the prescribed guidelines, were followed at NTI. Viability testing of these isolates is also being performed in our Bacteriology section before making them available for animal experiments. From a list of 180 isolates with ten different profiles of drug resistance, three isolates from each profile will be tested. Accordingly, 30 coded isolates based on the objective requirements will be used for the study.

B. Guinea Pigs:

Homogeneous stock of Albino Guinea Pigs that are bred and raised at NTI will be utilized during the course of study. Animals will be fed on a palleted concentrate diet and green lucerene besides providing boiled & cooled water ad-lib. Randomly selected animals from the same generation, weighing 250-450 Grams, keeping in view different time point experiments, will be used for this study. Animals of both sexes will be kept in pairs of same sex in pre-labeled stainless steel cages on the designated racks of animal isolators. Each animal will be identified by means of tags/ number tattooed on the ear as per the established procedures (8). As per the study requirement ten plus two (control) animals for each coded isolate to be tested will be used. Accordingly, a total of 360 NTI-bred albino guinea pigs will be utilized during the experimentation.

***C. Animal Inoculation*:**

For each coded strain ten animals 10 + 2 animals will be injected 0.5 ml suspension containing 1 mg of culture suspended in sterile phosphate buffer solution (PBS) / Placebo through subcutaneous route in the medial aspect of the left thigh. Two animals will be given placebo as controls for each coded isolate. The subcutaneous route of infection will be used as it is the closest to the aerosol route in terms of deposition of bacilli in a defined anatomical site, followed by local growth, progressive dissemination through lymphatics into the circulation and eventually the hematogenous seeding of other organs to multiply and produce gross disease in the spleen, liver & lungs (9). Moreover, irrespective of higher bacillary load required for subcutaneous inoculation the disease produced in the guinea pig animal model for TB is similar to widely accepted aerosol route (10).

D. Necropsy Procedure:

Guinea pigs from each group will be sacrificed using at different time intervals, as per the specific objectives of the protocol ranging from 2 to 48 weeks post- infection along with the required control animals that will be given placebo. Post-mortem examination will be carried out immediately after death. The body weight of each animal will be recorded before infection, weekly during post-infection and just before sacrifice. The animals will be subjected to dissection by the trained staff of AMRU. Gross lesions of the target organs namely spleen, liver, lungs and lymph glands will be scored by an independent reader following the standard method as described in our previous published studies (10&11).

E. Colony Counts on Spleen Homogenates:

Separate sterilized Teflon-glass tissue grinding tubes containing 5/4.5 ml of gel-saline will be used for each spleen for mechanical homogenization. The number of colony forming units (cfu) will be determined by inoculating serial ten fold dilutions of each homogenate on L-J media as per the standard procedures described in our earlier studies (11&12). Colonies of *M.tuberculosis* will be counted on 28th day of incubation at 37 C by two independent readers.

F. Design & Statistical Analysis:

Experiment with complete randomized design layout and blinded procedures will be followed according to the earlier published studies (9&13). Briefly the data generated from the animal experiments will be subjected to analysis of variance (ANOVA) for each group besides non-parametric Mann-Whitney test and other statistical techniques which will be obtained from the computer using SPSS/PC+4.0 software packages etc., in consultation with concerned staff of our Statistical section.

III. RESULTS

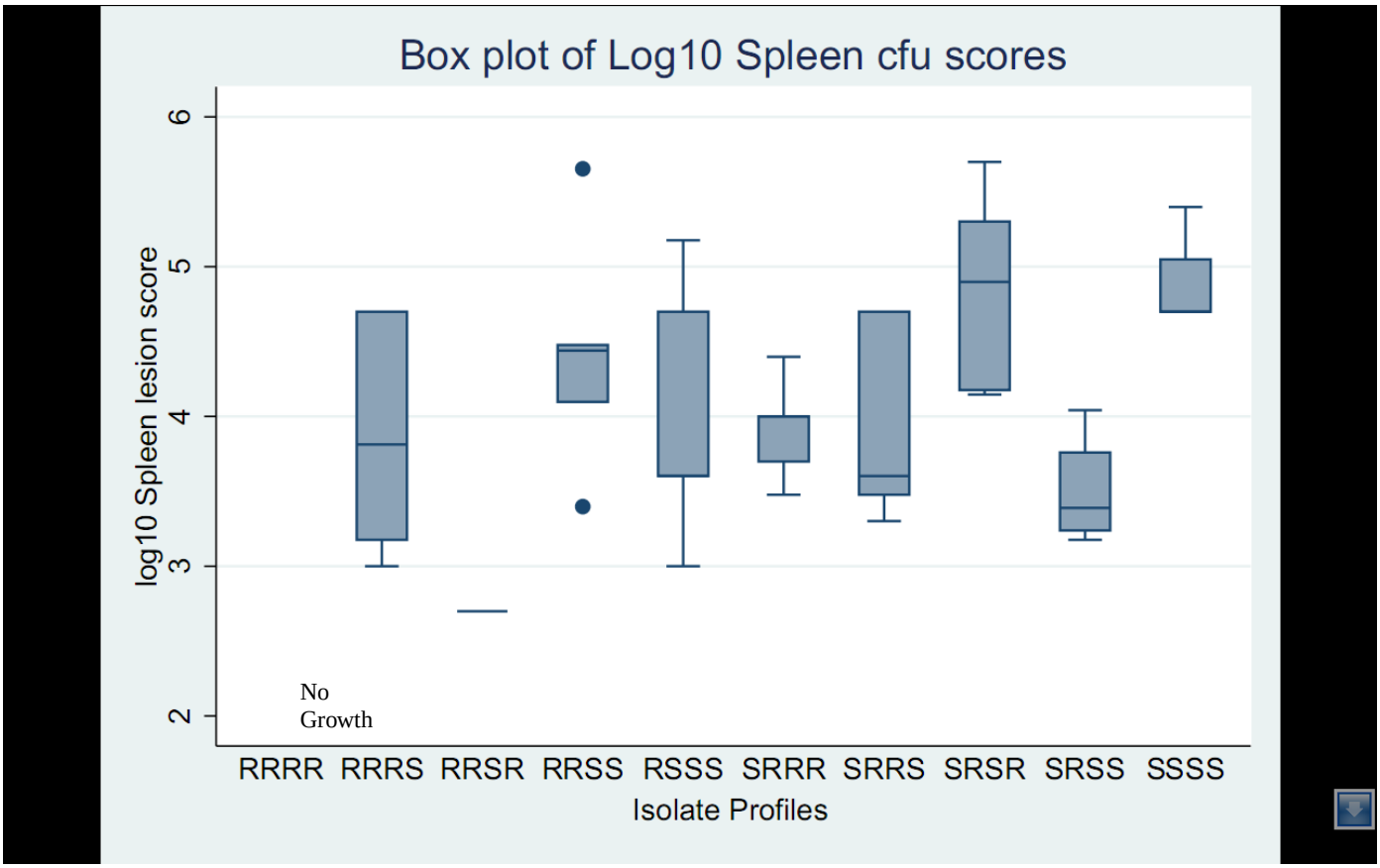

Figure 1:

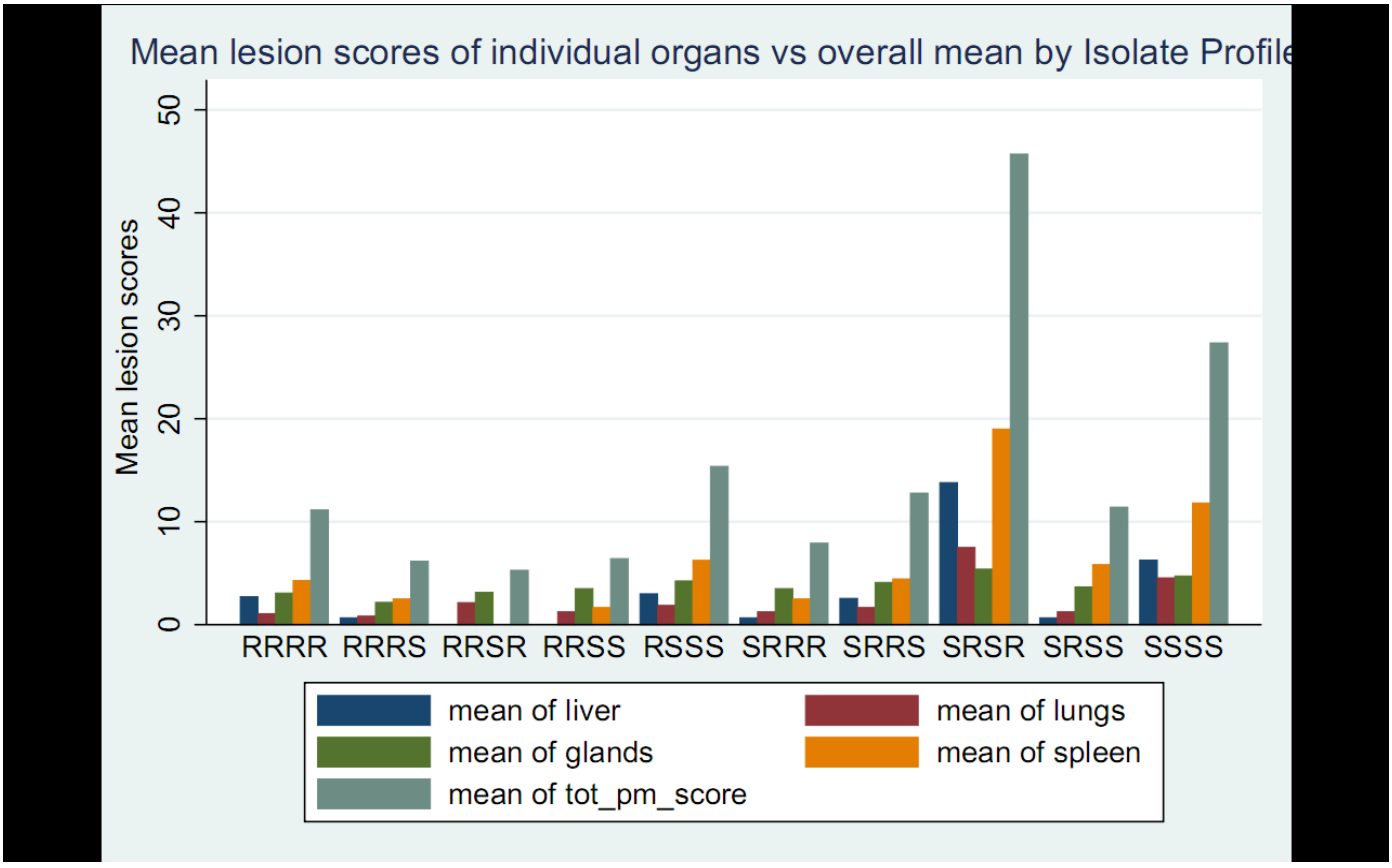

Figure 2:

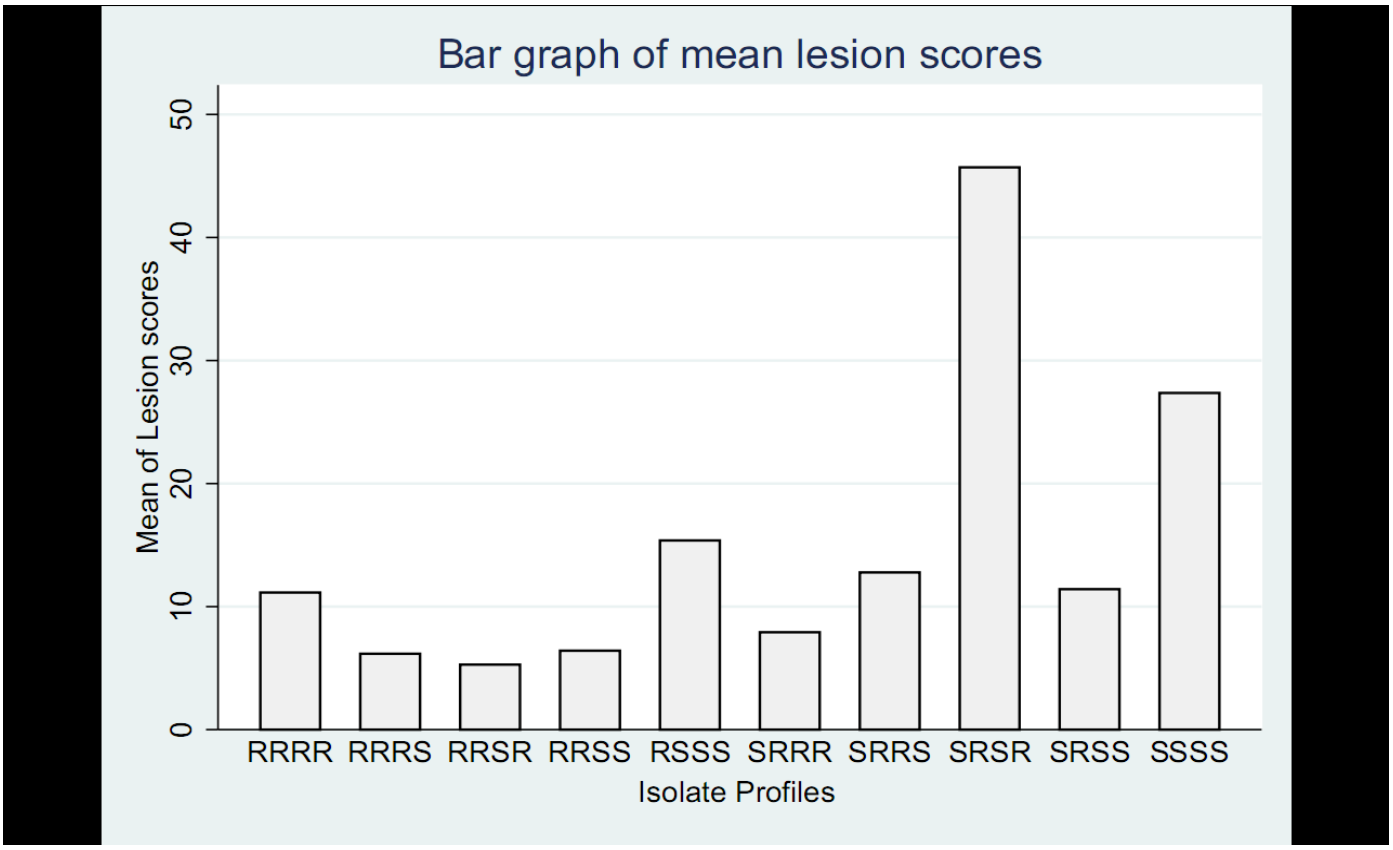

Figure 3:

IV. DISCUSSION

All cultures used in this study and recovered from the spleen tissues of the guinea pigs, preserved in deep-freeze, may also be useful for other investigators interested in further research work, aimed at developing innovative intervention strategies to combat the implications of drug-resistant TB in precise and most effective way.

CONCLUSION

Based on the tuberculous lesions as shown in the Figure-1, among ten drug-susceptibility profile groups SRSR group produced significantly extensive disease ($p<0.05$) except all drug sensitive SSSS group. Further results as in Figure-2&3 confirmed organ wise severity of disease based on the lesion scoring method by the independent readers. These findings however need to be done on larger samples collected from across India with additional inputs on genome of emerging mutants.

ACKNOWLEDGEMENT

This paper is dedicated to my beloved teacher late Professor Dr. Donald W Smith, University of Wisconsin, Madison, USA. His contribution in experimental TB research will continue to inspire scientists, for fulfillment of gaps in our knowledge about the tubercle bacilli besides facing future challenges. Most of the equipments used in our in vivo experiments were gifted by him being associated in our earlier in vivo experiments. We wish to thank our Technical Co-ordination Committee and the Institutional Animal Ethics Committee for their valuable inputs. Special thanks to our other team members namely B.R. Thimmegowda, S. Tajunisa, H.D. Surendra, Jitendra Suryamshi and last but not the least all our Animal/Laboratory Attendants who too were helpful in this study. We remain indebted to the sacrifice of guinea pigs for their enormous contribution in Bio-Medical Research.

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