

## INTER AND INTRASEROTYPE VARIATIONS OF STREPTOCOCCUS PNEUMONIAE PATHOGENICITY IN MICE AND RABBITS

### ABSTRACT

The small mammalian laboratory animals, the mice and the rabbits were tempted to be pathogenicity models for *S.pneumoniae* serotypes 1 and 6, the infecting doses were 0.3ml for mice and 0.5 ml for rabbits. These infectious doses were rated to a count of  $1 \times 10^7$  to  $7 \times 10^7$  CFU/ml. The infection routes were intraperitoneal for mice and intranasal for rabbits. The gold standard criteria for pathogenicity were the death end point DEP. DEP was up to 24hr for mice and ranged from four to 14 days in rabbits. Mice were more susceptible than rabbits for *S.pneumoniae* serotypes infections. Autopsy cultures of blood, lungs, liver and kidneys were producing same infecting serotype both from mice and rabbits. This isolation profile was indicating bacteremic state induced by infection. Different serotypes have shown different death end points duration time. There were inter and intraserotype variations in DEP. Moderate to severe respiratory disease conditions RDC was noted in mice while mild RDC in rabbits. Both of the tested models were similar lung histology to man. The histological features of this experimental infections have shown that the infected mice and rabbits were of bronchopneumonia type. It appears that both mice and rabbits were standing as valid laboratory animal models for these local clinical isolates of *S.pneumoniae* serotypes.

### KEY WORDS

Capsule, death endpoint, pneumococcal disease, bacterial virulence, respiratory disease, serotype variations

### INTRODUCTION

Bacterial eco-pathotype was showing variations in pathogenicity to laboratory animal models within the same pathogen species and /or serotypes. A theme proved right now both in man and small mammalian laboratory animals. Host health state, age, nutrition, genetic makeup, and other environmental insults do affect variations in the pathogenic potentials of human pathogenic bacteria. These influences are smoothly true for the pathogenicity of *S.pneumoniae* in man and laboratory animals. *Streptococcus pneumoniae* pathogenicity in man and laboratory animals are mediated by either one or more of the following; Pneumolysin and IgA splitting enzyme, bacterial subunit structures like capsules, bacterial-host body immune reactions, serotype phase variations and virulence genes [1-10]. The objective of the present work was to report on inter and intraserotype variations in pathogenicity of *S.pneumoniae* in mice and rabbits.

### MATERIALS AND METHODS

1- *S.pneumoniae* :

Pneumotropic clinical isolates of *S.pneumoniae* serotypes were constituting the first author collection. They were revived from the storage media, in liquid enriched media and

recharacterized as gram positive cocci mainly in pairs, alpha hemolysin producers, bile solubility positive, optochin positive, inulin fermentation. Positive results in KBOOBHISTrep Kit India and serotyped by DENKA SEIKEN CO. LTD [9, 10, 11].

### 2-Mouse Pathogenicity Test:

A volume of 0.3 ml of a broth culture from *S. pneumoniae* containing  $1 \times 10^7$  CFU/ml. was injected intraperitoneally into mice. The injected mice were observed for the onset of respiratory signs over six hours. Dead mice were eviscerated [11]. Blood, lung, liver and kidney were collected both for re-isolation studies onto Gentomycin blood agar, growth were identified as in [12], and for histologic sections. Tissue blocks were processed for histology tissue sectioning and staining [13].

### 3-Rabbits Pathogenicity:

A 24 hours Tryptone Soy 5ml fresh broth culture was centrifuged at 2500 rpm for three minutes. Pellets were washed with one ml saline. Cell counts were adjusted to  $1 \times 10^7$  CFU/ml. Then, 0.5 ml doses were inoculated intranasal in rabbits in an upright position for 5 minutes. Inoculated rabbits were observed for the onset of respiratory signs over 24 hrs. post to the infection [14]. The diagnostic infection criteria for pneumonia were; body temp, movement, mouth foam, and breath rate, histologic section as in [13], re-isolation studies [15].

## RESULTS

The onset of respiratory signs (PS) and the death end points (DEP) in mice and rabbits were indicating that mice were more vulnerable to infection with *S. pneumoniae* than rabbits, Table -1. DEP for mice were up to 24 hours while that of rabbits were ranging from 4 to 14 days. There were interserotype, Table -2 and intraserotype, Table -3 variations in pathogenicity of *S. pneumoniae* serotypes both in mice and rabbits. Mice have shown moderate to severe while rabbits have shown mild forms of respiratory disease. Applying Koch's postulates on infected mice and rabbits, findings in Table- 4 were confirming the pathogenicity of *S. pneumoniae* to man. Both of the gross, histology, Table - 5, Fig. - 1 and Figure-2. The sections showed suppurative neutrophilic cell infiltration in lung parenchyma, alveoli, and intrabronchial lumen. These tissue reactions are consistent with bronchopneumonia rather than pneumonia. Re-isolation studies of the infections in mice and rabbits were indicating that these animals reaching bacteremic state of infection since the bacteria have been re-isolated from blood, lungs, and liver. Hence they are valid models simulating bronchopneumonia in man.

Table- 1 : Interserotype variations in *S. pneumoniae* in mice and rabbits

Serotype	Mice/IP/0.3ml. $1 \times 10^7$ DEP in hours	Rabbits/0.5ml./ $1 \times 10^7$ DEP in days
1	12	4
2,6,8	18-20	6-8
7	24	14

Table- 2 :Intraserotype variations in pathogenicity of *S.pneumoniae* serotype 6 in mice and rabbit.

Number of isolates	Mice/IP/0.3ml 1x10 to 7DEP in hours	Rabbit/IN/0.5ml 1x10 to 7,DEP in days
1	18	6
3	20	8
3	24	10
1	24	14

Table – 3 : Pathogenicity spectrum of *S.pneumoniae* serotypes in mice and rabbits.

Serotype	Disease in mice	Disease in rabbits
6	Moderate respiratory disease condition	Mild respiratory disease condition
1,2,8	Moderate to severe respiratory disease condition	Mild respiratory disease condition
3,4,5,7	Mild to moderate respiratory disease condition	Mild respiratory disease condition

Table – 4 : An autopsy reisolation culture studies for mice and rabbits infected with Serotype 1 and 6

Infected animal	Blood	Lung	Liver	Kidney
Mice; 1 ,6	+	+	+	+
Rabbit; 1 , 6	+	+	+	+

Table-5 :Histologic features of mice and rabbit lungs infected with *S.pneumoniae* serotype 1.

serotype	Animal	Dose	DEPduration time	Histologic features	Conclusion
1	Mice	0.3 ml,IP,1x10 to 7 CFU/ml.	12 hours	Neutrophilic infiltration in lung paranchyma,	Bronchopneumonia

				aveoli and intrabronchial inflammatory cells infiltration as compard to normal tissue contour in control	
1	Rabbit	0.5 ml ,intranasal,1x10 to 7 CFU/ml.	4 days	Neutrophilic infiltration in the lung paranchyma ,alveoliand ,intrabronchial inflammatory cell infiltration and debries. Aa compared to normal tissue contour in controls.	Bronchpneumonia

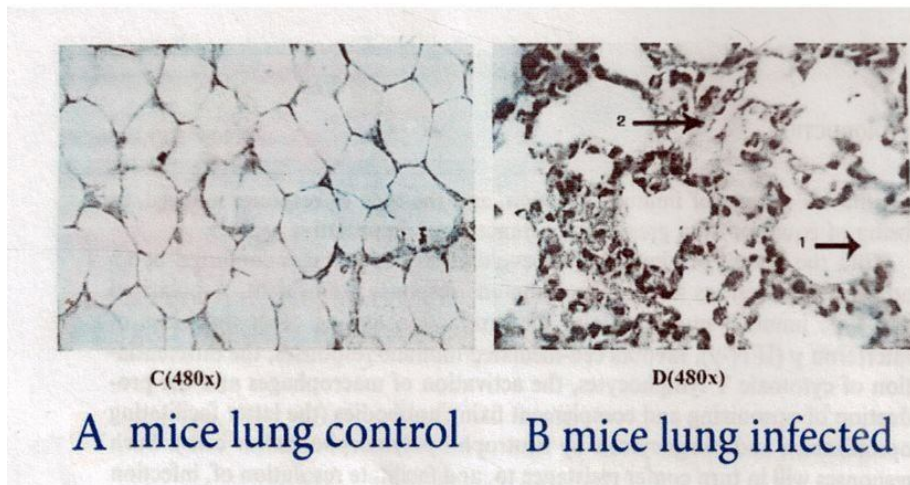
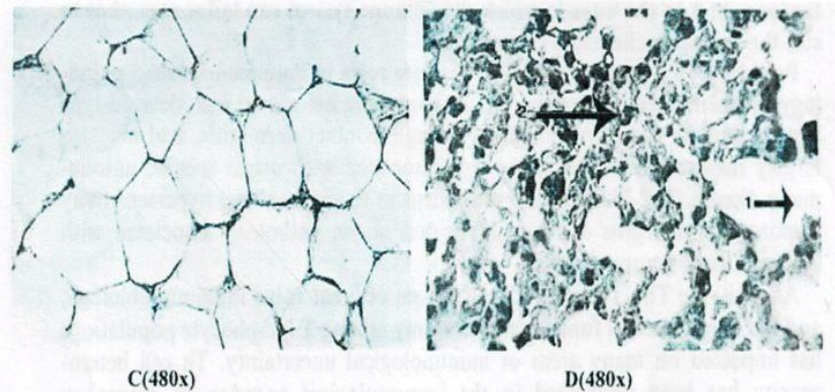


Figure – 1 ; A-normal mice lung tissue architecture B-Infected mice lung tissue showing 1- Inflammatory cell infiltrate in lung tissue parenchyma ,2- Intrabronchial inflammatory cell infiltrate. 1 and 2. Consistent with bronchopneumonia..

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**A Rabbit lung control B Rabbit lung infected**

**Figure – 2 :** A Normal rabbit lung tissue architecture B-infected rabbit lung tissueshowing 1 – Inflammatory cell infiltrate in lung tissue paranchyma,2-Intra-broncheolar inflammatory cell infiltrate.1 and 2 consistent with bronchpneumonia.

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## DISCUSSION

Digging through the current literature all over the world have shown that *S.pneumoniae* does have multiple pathoserotypes[1-9].There were serotype dependent pathogenic potentials[3,4,5].The present communication was tempting to uncover an inter and intraserotype variations in pathogenicity to mice and rabbits Tables- 1-3.Mice have shown pneumonic disease similar to that of man with moderate to severe disease forms.The DEP of mice were 12 to 24 hours.While that of rabbits were ranging from 4 to 14 days.Such pathogenicity spectrum is rather similar to that of man[14].Serotype -1 was reported to be the most virulent,Table- 5 , than other serotypes this was inline with results of other workers.But serotype 6 was of low virulence[17-21].Infection of mice and rabbits with *S.pneumoniae* were either producing lobar pneumonia or bronchopneumonia[20,21,22], results in table 5 Fig- 1 was indicating bronchpneumonia.Results in this experimental settings are being novel in this area.

## CONCLUSIONS

Different *S.pneumoniae* serotypes have shown different pathogenicity spectrum. There were an inter and intraserotype variations in pathogenicity to mice and rabbits as indicated by DEP duration time. Disease picture rather simulating that of man. Mice and rabbits were proved as valid laboratory animals for pathogenicity of this pathogen in human.

#### Ethical Approval :

1-The care, housing, handling and interventions on both rabbits and mice were done following the international acts regulating the care, housing, handlings, and interventions.

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