Induction & resolution of lobar pneumonia following intranasal instillation with *Klebsiella pneumoniae* in mice

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**Background & objectives:** Pneumonia caused by *Klebsiella pneumoniae* is important due to its high morbidity and mortality, especially in context of nosocomial infections. Many experimental studies have focused on the induction and progression of infection till it peaks, but the process of resolution has not been described. In the present study, we successfully attempted to establish an acute respiratory tract infection model in BALB/c strain of mice with *K. pneumoniae* employing a simple, reproducible intranasal instillation method.

**Methods:** Experimental pneumonia was induced by two strains of *K. pneumoniae* in BALB/c mice following intranasal instillation, and the course of pneumonia was studied by bacteriological and histopathological evaluation of the lung tissue.

**Results:** Both the strains were similar in their ability to induce infection which peaked on day 3, post infection. However, a strain dependent difference in relation to bacterial load and the process of resolution was observed.

**Interpretation & conclusion:** The present study provides a model of lobar pneumonia produced by *K. pneumoniae* which can be useful for studying therapeutic and preventive interventions.

**Key words** *Klebsiella pneumoniae* - lobar pneumonia - nosocomial infections - respiratory tract infections

*Klebsiella pneumoniae* is an important cause of both community acquired, as well as nosocomial lung infections. Pneumonia caused by this organism has a rapidly progressive clinical course which is often complicated by multilobular involvement and lung abscesses1,2, which leaves little time to institute effective antimicrobial treatment. As a result, the mortality rates may reach or exceed 50 per cent even in treated cases3-6. The populations at risk are neonates, immunocompromised individuals, and patients predisposed by prior surgery or malignancy7,8. An increase in the populations at risk for developing pneumonia, and an increase in the emergence of multidrug resistance among *K. pneumoniae* nosocomial isolates9 has renewed interest in the investigation of alternative approaches for the treatment, and prophylaxis of respiratory tract infections due to *K. pneumoniae*.

To study the therapeutic or prophylactic potential of any molecule, a suitable animal model simulating as far as possible the natural course of infection, is required. Earlier studies have shown that pneumonia can be induced following introduction of the pathogen by the intratracheal10, as well as intranasal11 route. Intratracheal...
instillation method which involves surgical intervention, also requires exposure of the respiratory tract. Most respiratory tract infections are contracted through aerosol inhalation, and therefore a model making use of this route for induction of infection would simulate the natural route of infection. Yokota et al\textsuperscript{12} reported an aerosol inhalation model but this model had a low infection take rate, and poor reproducibility. In a pilot study, employing aerosol inhalation our observations were similar which required an alternate approach for inducing experimental pneumonia.

The findings of studies on the induction of pneumonia following intranasal instillation are conflicting. While some workers\textsuperscript{11,13} have reported success in inducing pneumonia by intranasal instillation others\textsuperscript{14} could not induce infection with this method. Most studies focused on the induction and progression of infection till it reached the peak, but the process of resolution was not studied. In the present study, we have attempted to establish an acute respiratory tract infection model in BALB/c strain of mice with \textit{K. pneumoniae} inducing lobar pneumonia, and its resolution over a period of 10 days.

### Material & Methods

**Bacterial strains:** Two strains of \textit{K. pneumoniae} ATCC 43816, (obtained from Dr David P. Speert, Department of Paediatrics, University of British Columbia, Vancouver, Canada) and B5055 (obtained from Dr Mathia Trautman, Department of Medical Microbiology and Hygiene, University of Ulm, Germany) were used in the study. Both the strains were identified as \textit{K. pneumoniae} using standard procedures\textsuperscript{15}.

**Bacterial inoculum:** Bacterial strains maintained on nutrient agar slant, were grown in static culture in nutrient broth at 37°C for 18 h. Organisms were harvested by centrifugation at 2348 g for 15 min, washed three times, and suspended in phosphate buffered saline (PBS, 0.2 M, pH 7.2) to the desired concentration.

**Animals:** BALB/c strain of mice procured from the Central Animal House, Panjab University, Chandigarh, weighing 20-30 g were used for the study. They were fed on standard antibiotic free synthetic feed (JDB Agencies Private Ltd., India). All the experiments were carried out at the Department of Microbiology, Panjab University, Chandigarh. The study protocol was approved by the institutional ethical committee for animal experimentation.

**Standardization of the bacterial inoculum for induction of pneumonia:** The optimal dose required for establishing pneumonia in mice was standardized prior to studying the course of pneumonia. For this, doses ranging from 10\textsuperscript{2} to 10\textsuperscript{6} cfu/ml were given intranasally. A group of 15 mice were infected with each dose. Mice were sacrificed on day 3 post infection (PI) to check for bacterial load in the lung tissue, and calculation of infection take rate. A dose that gave 100 per cent infection take rate without causing any mortality was taken as the optimal dose.

**Induction of pneumonia by intranasal route:** For intranasal instillation of the bacterial inoculum, the method of Held et al\textsuperscript{11} was employed. 50 µl bacterial inoculum was instilled into the nasal opening while holding the mouse upright. Thirty mice were infected in each set of experiment (done three times). Groups of 5 mice were sacrificed by cervical dislocation on days 1, 2, 3, 5, 7 and 10 PI. The Viscera of mice were exposed after thoroughly cleaning the area with alcohol. Lungs were removed aseptically and were examined for bacterial counts and histopathology.

**Bacteriological examination:** Lung tissue was sectioned into two halves. One half of each lung was placed in a sterile tube and weighed. The tissue was homogenized in a Corning glass homogenizer with 1.0 ml of sterile PBS (0.2M, pH 7.2). Serial dilutions of the homogenized mass in PBS were plated on MacConkey agar plates (Hi-Media, Mumbai) in triplicate. Plates were incubated at 37°C for 24 h, and the viable counts were determined.

**Histopathological examination:** Lung tissue preserved in formalin was dehydrated in ascending series of alcohol (70-100%). The tissue was embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (Hi-Media, Mumbai). Grading of the severity of pathological lesions was also done. For evaluation, a section of each lung was assessed on a semi quantitative scale of 0 to 3 (Table). A total score indicative of the overall severity of lesions was determined by adding the individual score.
Fig. 1. Bacterial counts of lung tissue homogenate following infection with *K. pneumoniae* B5055 (10⁴ cfu/ml) and 43816 (10⁴ cfu/ml) in BALB/c strain of mice (each value is mean ± SD of 3 replicates, *P*<0.01).

**Table.** Semi quantitative scores for grading the severity of pathologic lesions of the lungs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Histologic changes</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Alveoli</td>
<td>No change</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oedema</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>Inflammatory cells in the alveolar lumina</td>
<td>+2</td>
</tr>
<tr>
<td></td>
<td>Inflammatory destruction of alveoli</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td>(lung abscess)</td>
<td></td>
</tr>
<tr>
<td>Bronchioles</td>
<td>No change</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mild inflammation in the wall</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>(without luminal slough)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe inflammation in the wall</td>
<td>+2</td>
</tr>
<tr>
<td></td>
<td>(with luminal slough)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe inflammation with luminal slough</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td>and peribronchial inflammation</td>
<td></td>
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</tbody>
</table>

**Capsular polysaccharide (CPS) estimation:** CPS content was estimated by the method of Blumenkrantz and Asboe-Hansen.¹⁶

**Statistical analysis:** Student’s ‘t’ test was used to compare bacterial counts and histopathological scores at different days post infection.

**Results**

For *K. pneumoniae* B5055, a dose of 10⁴ cfu/ml given intranasally was found to be optimal for inducing pneumonia. Infection was established after 24 h of exposure to this dose. Bacteriological findings at various time intervals showed that the lung bacterial load was highest on day 3 PI, followed by a significant decrease on day 5 and 7 PI (*P*<0.01). Lungs became sterile by day 10 PI (Fig. 1). Histopathological analysis of the lung...
tissue revealed that following 24 h of exposure to *K. pneumoniae* B5055, mice developed mild pneumonia (score 1-2), characterized by cellular infiltrate composed of neutrophils and a few macrophages. Lungs of mice sacrificed on day 2 PI revealed moderate changes (score 2-4). While on day 3 PI, animals showed well-developed pneumonia with abscess formation and destruction of alveoli (score 4-6, $P<0.01$) (Fig.2a-c). Histopathology of mice sacrificed on day 7 PI showed resolving pneumonia and macrophages dominating in the affected areas (Fig.3a-b).

For the strain 43816, a dose of $10^4$ cfu/ml was found to be optimal for induction of infection. The course of establishment of pneumonia in mice following exposure to this dose was similar to that observed for the strain B5055 (Figs 1 and 2d). The only difference being that 43816 persisted till day 10 PI. However, the histopathological picture was the same for both the strains showing resolution of infection after day 7 PI. No significant differences were observed in the CPS production by the two strains (37 µg uronic acid/ml for B5055 and 42 µg uronic acid/ml for 43816).

**Discussion**

Respiratory tract infections all over the world are one of the most common and severe form of infections treated by health care practitioners\(^1^7\). Further in this context, hospital acquired pneumonia is the second most common infection causing high morbidity and mortality\(^1^8\). *K. pneumoniae* alone accounts for 25-43 per cent of the nosocomial pneumonias caused by Gram-negative bacteria\(^1^9\). Importance and magnitude of the problem, therefore, requires concerted efforts of the investigators for developing effective preventive and curative strategies.

In an earlier study carried out by Brendt et al\(^1^3\), pneumonia was induced following intranasal inoculation by *K. pneumoniae* A-D type 1 strains in F-344 rats. Subsequently some workers\(^1^0,2^0\) have used mice with infection being introduced intratracheally. This method of infection however, has limitations in terms of extrapolation of data to natural infection in humans. In a recent study,
Both the strains employed in the present study were of capsule type 2, which is the most common capsular type isolated from patients with pneumonia, urinary tract infections or bacteraemia. In spite of the similarity of the capsular type and no quantitative difference in the CPS production, a significant difference was observed in the clearance of the two strains from the lungs of the experimental animals. CPS, a well recognized virulence factor, therefore, does not seem to have contributed to the difference observed. Earlier *K. pneumoniae* 43816 has been reported to induce a marked inflammatory response. On one side the process of inflammation is supposed to help the host, but excessive and persisting inflammation can do damage to the underlying tissue which may interfere with pathogen elimination. It however remains to be seen as to which other virulence factor(s) could have helped 43816 to survive and persist for a longer duration *in vivo* in the lung tissue. Further, the host factors such as cytokine response, free oxygen radical generation and nitric oxide production may also play a role in differential virulence of the organism. Studies on these lines are in progress in our laboratory.

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**References**


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