

## Lobar Pneumonia in Rats Produced by Clinical Isolates of *Klebsiella pneumoniae*

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Transtracheal instillation of clinical isolate *Klebsiella pneumoniae* serotype 1 (KP1) into the lungs of rats resulted in the production of a characteristic, chronic lobar pneumonia. To further examine this phenomenon, two variants of this organism were employed in this experimental model. These variants differed markedly in capsule size, colony morphology, and in virulence, as determined by mouse lethality tests. The ability of these strains to establish a lobar pneumonia in rats correlated with the virulence of the respective organisms as monitored by intraperitoneal injection in mice. The 50% lethal doses in mice were  $4.9 \times 10^1$  colony-forming units (CFU) for the more virulent KP1 strain (KP1-O) and  $1.42 \times 10^5$  CFU for the less virulent variant (KP1-T). In the rat lung model, marked lung pathology was evident by day 6 with a KP1-O inoculum of  $5 \times 10^2$  CFU, whereas KP1-T caused little or no lung pathology when delivered transtracheally at a concentration of  $7 \times 10^6$  CFU. Two relatively nonvirulent variants of *K. pneumoniae* serotype 2 were also used in this rat lung model and were found not to produce a lobar pneumonia even when delivered in large doses. These results indicate that a chronic lobar pneumonia can be established in a rat model if the appropriate organism is employed and the virulence of *K. pneumoniae* injected intraperitoneally into mice is an excellent indicator of an organism's potential to cause lobar pneumonia in rats.

The prolonged survival of chronically and critically ill patients, due to the increased quality of medical care in this country has been paralleled by a striking increase in the occurrence of gram-negative bacillary infections (23). Hospital-acquired pneumonias caused by these organisms have increased to the point where they comprise nearly 50% of all nosocomial pneumonias (13). One gram-negative bacterium, *Klebsiella pneumoniae*, accounts for 25 to 43% of gram-negative nosocomial pneumonias, thus making it the most common agent in this disease process (29). Pneumonia caused by *K. pneumoniae* is particularly dangerous because, once it has been established, it is extremely difficult to control (13, 19), and mortality rates may reach or exceed 50%, even in treated cases (15, 19).

To study the bacteriological factors associated with lower respiratory tract disease caused by *K. pneumoniae*, we felt that a good animal model of lobar pneumonia was necessary. The low price and ease of maintaining small rodents make them appealing animals for these kinds of studies. However, Berendt et al. (3) reported that intranasal inoculation of *K. pneumoniae* into rats resulted in bronchopneumonia rather than a lobar pneumonia. Intratracheal instillation of *K. pneumoniae* has been reported by Sale

and Wood (27) to produce a lobar pneumonia in rats, but these authors found it necessary to use gastric mucin, a procedure which may have untoward effects on the animals (2). We have been successful in establishing a lobar pneumonia with *K. pneumoniae* in rats without the use of mucin. The pathology that we observed closely resembled that seen in the human form of the disease. In this study we demonstrate that the strain of *K. pneumoniae* used to establish a lung infection is very important, as not all strains are capable of producing a lobar pneumonia in rats. We also demonstrate that the virulence of various strains of *K. pneumoniae* in mice is directly related to their ability to produce a lobar pneumonia in rats.

### MATERIALS AND METHODS

**Bacteria.** *K. pneumoniae* serotype 1 (KP1) (lung isolate, strain 2083, American Type Culture Collection, Rockville, Md.) and *K. pneumoniae* serotype 2 (KP2) (blood isolate, ATCC 209011) were grown in Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) and stored at  $-20^\circ\text{C}$  in 20% glycerol in TSB. Stock cultures were thawed, and 0.1 ml was inoculated into 50 ml of the appropriate media (described below) and incubated at  $37^\circ\text{C}$  overnight at 200 rpm in a shaking water bath. A 0.1-ml amount of this culture was added to 50 ml of fresh medium and

grown to mid-logarithmic phase (absorbance equal to 0.1 at 550 nm). A 20-ml amount of the resulting suspension was then centrifuged at  $10,000 \times g$  for 20 min at 4°C, washed twice with phosphate-buffered saline (PBS), and suspended in a final volume of 20 ml of PBS. Colony counts were performed on Trypticase soy agar (TSA) (BBL) for each experiment, using routine plating procedures. Identification was confirmed by both the API 20E system (Analytab Products, Plainview, N.Y.), and type-specific antisera (Difco Laboratories, Detroit, Mich.).

Colony morphology variants were noted in the KP1 and KP2 population on TSA plates. Two major colony types were observed in both strains, an opaque (O) and a translucent (T) variant. These subtypes bred true and have been previously described in other *K. pneumoniae* populations (8, 18). The four variants utilized in this report were thus designated KP1-O, KP1-T, KP2-O, and KP2-T.

**Media.** Two different media were used in these studies. Organisms were grown either in TSB or in a chemically defined medium. The defined medium was composed of the following: 85.6 mM NaCl, 92 mM sodium glutamate, 70 mM glucose, 1.33 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.14 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.0085 mM  $\text{ZnSO}_4$ , 0.0039 mM  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 18.37 mM  $\text{KH}_2\text{PO}_4$ , and 43.06 mM  $\text{K}_2\text{HPO}_4$  in a liter of deionized and distilled water. The pH was adjusted to 7.1, and the media were filter-sterilized with a 0.45- $\mu\text{m}$  filter.

**Test animals.** Male Sprague-Dawley rats weighing 200 to 250 g and male Swiss-Webster mice weighing 15 to 20 g were used in these studies. Both were housed in plastic cages in groups of four (rats) or five (mice), and both had access to commercial chow and water ad libitum.

**Animal inoculation.** Rats were lightly anesthetized with ether, and the ventral cervical region was cleaned with a 95% alcohol rub. A 0.5-in (ca. 1.27 cm) medial longitudinal incision was then made in the animal to expose the trachea. The trachea was incised, and 0.05 ml of a washed suspension of various concentrations of *K. pneumoniae* was placed into the left, diaphragmatic lobe of the lung via a bead-tipped, curved inoculating needle. The incisions were allowed to heal of their own accord, and rapid closing (24 h) was noted without evidence of infection.

**Tissue sampling and preparation.** At various intervals after inoculation, groups of rats were exsanguinated by cardiac puncture under ether, the thoracic cavity was opened, and the lungs were aseptically removed. The lungs were weighed and then used for either bacterial quantitation or for histological examination. Samples of lung tissue were excised from affected areas and fixed immediately in 10% Formalin. Samples were cut at 4  $\mu\text{m}$  and stained with hematoxylin and eosin on Brown and Haup stains. The sections were examined microscopically, and photomicrographs were made from representative areas.

For bacterial quantitation, lungs were homogenized in 5 ml of sterile phosphate-buffered saline (PBS) at 4°C with a Brinkman polytron homogenizer (Brinkman Inst.). Serial 10-fold dilutions were made from the homogenate, and 0.1 ml of selected dilutions was plated out on TSA and incubated overnight at 37°C. The following day, colonies were counted, and concentrations of *K. pneumoniae* in lung specimens were calculated. Lung bacterial counts were calculated as

the total number of bacteria present in an entire lung specimen and were reported as the total bacterial count (TBC) per set of lungs. For convenience the TBC was expressed in logarithmic units to the base ten ( $\log_{10}$  TBC).

To determine the 50% infectious dose ( $\text{ID}_{50}$ ) of organisms used in these studies, rats in each group with TBC values equal to or exceeding  $5 \times 10^4$  colony-forming units (CFU) ( $\log_{10}$  TBC = 4.70) at day 6 post-inoculation were considered infected. Infected rats were counted, and the  $\log_{10}$   $\text{ID}_{50}$  was calculated by the method of Reed and Muench (25).

**Mouse lethality tests.** Fifty percent lethal dose ( $\text{LD}_{50}$ ) studies were performed in mice with each of the various *K. pneumoniae* strains. Mice were divided into five groups of five mice each, and each group received intraperitoneally 1 ml of one of five serial 10-fold dilutions of the freshly prepared, mid-logarithmic-phase cultures in sterile PBS. Dead mice were counted and removed from their cages at 24-h intervals.  $\text{LD}_{50}$  were calculated after 72 h of observation by the method of Reed and Muench (25). The  $\text{LD}_{50}$  was also expressed employing  $\log_{10}$  units for comparison with  $\log_{10}$  TBC values from the rat studies. Each  $\text{LD}_{50}$  value reported is the average of at least two trials. Two-tailed Student *t*-tests (28) were performed to determine whether statistically significant differences existed between the  $\text{LD}_{50}$  values within each serotype.

**Serum lysozyme.** Blood obtained from rats was allowed to clot at 4°C overnight, and the serum was obtained by centrifugation. Lysozyme levels in serum were measured by the lyso-plate method of Osserman and Lawlor (22), using human urine lysozyme (Kallestad) as the standard. One-tailed Student *t*-tests (28) were performed for statistical analysis of the serum lysozyme data.

**Capsule size.** Capsule size was determined by the method of Duguid (6), using India ink preparations. Capsule production was expressed as the transverse diameter, which is a measure of both the width of the bacillus and the width of the capsule on either side of the soma. One hundred bacilli were randomly selected under oil immersion and measured with an ocular micrometer, and the average capsule size was calculated.

## RESULTS

**Establishment of a chronic lobar pneumonia.** In initial studies all experimental rats received  $5 \times 10^6$  CFU of KP1 transtracheally in a 0.05-ml volume of sterile PBS, while control animals received the same volume of sterile PBS in a similar manner. Eight experimental and two control rats were sacrificed on days 1, 3, 6, and 9 post-inoculation (groups 1 to 4, respectively). Lungs from four of the eight experimental rats and from one of the two controls on each day of sacrifice were processed for histology, whereas the remaining four experimental and one control rat had their lungs removed for bacterial quantitation. Four experimental and one control rat were also sacrificed on days 7, 14, 21, and 28 post-inoculation (groups 5 to 8, respectively), having also received  $5 \times 10^6$  CFU of KP1.

TABLE 1. Establishment of chronic KP1 pneumonia in rats

Group <sup>a</sup>	No. of animals dead/no. inoculated	Day sacrificed	Log <sub>10</sub> TBC <sup>b</sup> (CFU) (range)
1	0/4	1	6.51 (5.96–6.90)
2	0/4	3	7.02 (5.67–8.46)
3	0/4	6	6.32 (4.11–8.51)
4	0/4	9	6.91 (4.60–8.95)
5	0/4	7	9.47 (9.05–10.16)
6	0/4	14	6.20 (5.86–6.65)
7	1/4	21	3.84 (2.00–5.00)
8	2/4	28	3.19 (ND <sup>c</sup> –6.38)
Control	0/8	1, 3, 6, 7, 9, 14, 21, 28	ND <sup>c</sup>

<sup>a</sup> Rats were inoculated transtracheally with  $5 \times 10^6$  CFU of KP1 in 0.05 ml of sterile PBS and sacrificed on the days indicated. Controls received 0.05 ml of sterile PBS in the same manner. All surviving rats (except the four used for histological processing on days 1, 3, 6, and 9) were used for bacterial quantitation of lung tissue.

<sup>b</sup> TBC, Viable bacterial count per whole lung expressed in log<sub>10</sub> units from surviving rats.

<sup>c</sup> ND, None detected at  $10^{-2}$  dilution of lung homogenate.

Results of lung bacterial concentration are shown in Table 1. The TBC is expressed as the log<sub>10</sub> of the average lung bacterial concentration for the experimental animals in each group. As can be seen, the log<sub>10</sub> TBC remained elevated (range of 4.11 to 10.16) throughout the first 14 days of the study, whereas no KP1 were detected in the lungs of the control animals. Only one of the eight experimental rats in the 21- and 28-day groups cleared KP1 from their lungs. Mortality for the entire population was 5% (3/60), with all deaths occurring in the latter two groups.

By day 2 postinfection, nearly all experimental rats appeared acutely ill. Mucous secretions exuded from their eyes, and most exhibited short and rapid breathing. As the infection progressed, their coats became shabby, and considerable weight loss was obvious. Gross examination of the lungs showed involvement of one or more lobes, often affecting the entire lobe in a typical lobar distribution with the majority of the lesions noted in the lower left lobe. This observation demonstrated that we were indeed introducing the organisms into the left diaphragmatic lobe. The involvement was characteristically massive and voluminous, presenting as dull, greyish regions that released copious amounts of purulent exudate upon sectioning. Histological examination also supported the establishment of a lobar pneumonia in this rat lung model (Fig. 1a to e). The progressive destruction of lung tissue

continued up to day 28, when the study was terminated.

**Effect of KP1 dosage.** The next set of experiments was performed to determine the ID<sub>50</sub> for the KP1 strain in the rat model. KP1 was grown to a concentration of  $1.55 \times 10^8$  CFU/ml in TSB and washed three times in cold sterile PBS. Groups received 0.05 ml of a suspension of KP1 (Table 2). Twenty percent (6/30) of the rats died during this experiment, with half of these belonging to group 1. Lung weight and serum lysozyme levels are also seen in Table 2. Serum lysozyme levels, which are known to co-vary with the extent of infection (4), were elevated with respect to control values, achieving significance at the  $P < 0.01$  level in two of the groups. Due to the marked swelling during the infectious process, the weight of the lungs increased up to more than three times that of normal. Rats were considered to be infected if they either succumbed to the KP1-induced pneumonia or if a TBC of at least  $5 \times 10^4$  was found in the whole lung of those rats harboring the organism. An ID<sub>50</sub> for KP1 of  $1.55 \times 10^5$  CFU was thus obtained.

To rule out the effect of the medium in which the bacterium was grown as contributing to the virulence of the organism, a defined medium was used, and the effect of dosage of KP1 was repeated in the same manner as above. The ID<sub>50</sub> obtained using the defined medium was found to be  $2.22 \times 10^5$  CFU, which does not differ significantly from the LD<sub>50</sub> value obtained using TSB. Therefore, the effect on virulence of growing KP1 in TSB appears to be negligible.

**Effect of strain variation on the ability of *K. pneumoniae* to cause a lobar pneumonia and to produce a fatal septicemia in mice.** India ink preparations of a number of strains of *K. pneumoniae* revealed that not all bacilli of the same strain possess a similar-sized capsule. Two predominant capsule sizes were found within many of the strains. A closer inspection of isolated colonies on TSA revealed that two basic colony types existed within a given strain that corresponded to the capsular size differences seen under India ink. In particular, for the KP1 and KP2 strains utilized in this study, there existed an opaque (O) and a translucent (T) colony type. These co-variants were labeled KP1-O and KP1-T and KP2-O and KP2-T, respectively. In both cases the opaque variant possessed the larger capsule. KP1-O possessed a capsule with an average transverse diameter of 5.6  $\mu$ m, whereas KP1-T exhibited a transverse diameter of 1.8  $\mu$ m. KP2-O and KP2-T had transverse diameters of 1.8 and 1.4  $\mu$ m, respectively. Biochemical and serological typing was performed on all variants to confirm species and serotypes.

The LD<sub>50</sub> in mice for KP1, containing the

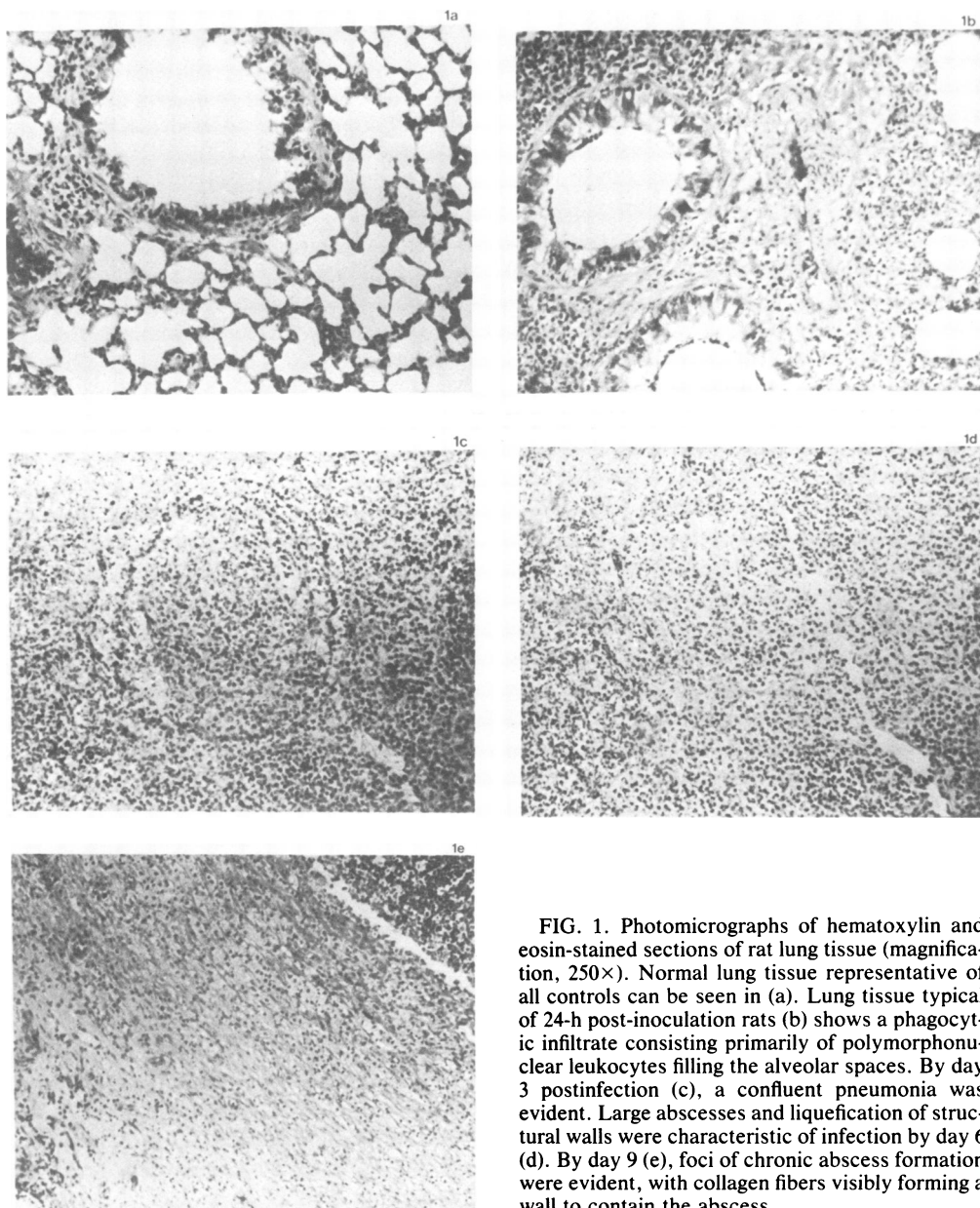


FIG. 1. Photomicrographs of hematoxylin and eosin-stained sections of rat lung tissue (magnification, 250 $\times$ ). Normal lung tissue representative of all controls can be seen in (a). Lung tissue typical of 24-h post-inoculation rats (b) shows a phagocytic infiltrate consisting primarily of polymorphonuclear leukocytes filling the alveolar spaces. By day 3 postinfection (c), a confluent pneumonia was evident. Large abscesses and liquefaction of structural walls were characteristic of infection by day 6 (d). By day 9 (e), foci of chronic abscess formation were evident, with collagen fibers visibly forming a wall to contain the abscess.

mixed population of KP1-O and KP1-T, was calculated to be  $1.92 \times 10^3$  CFU. When this strain was divided into its opaque (KP1-O) and translucent (KP1-T) variants and tested in the mouse model, it was found that KP1-O was a much more virulent organism than KP1-T. Table 3 summarizes the data obtained from the mouse virulence studies. KP1-O possessed an average LD<sub>50</sub> of  $4.99 \times 10^1$  CFU whereas KP1-T demonstrated an average LD<sub>50</sub> of  $5.34 \times 10^5$  CFU. Table 3 also shows the LD<sub>50</sub> values in mice for

KP2 and its variants. The mixed KP2 population gave an LD<sub>50</sub> of  $4.3 \times 10^5$  CFU but, when separated into its opaque (KP2-O) and translucent (KP2-T) variants, LD<sub>50</sub> values of  $1.8 \times 10^5$  and  $>6.2 \times 10^7$  CFU, respectively, were obtained. Thus for both strains, the organism with the larger capsule was also more virulent.

These strains, with the exception of the non-virulent KP2-T, were then examined in the rat lung model. All organisms were grown in defined medium and harvested as described above.

TABLE 2. Effect of dosage on the ability of KP1 to produce pneumonia in rats

Group <sup>a</sup>	No. of animals infected/no. inoculated <sup>b</sup> (deaths)	Wt of lung (g) (range)	Log <sub>10</sub> initial dose (CFU) <sup>c</sup>	Log <sub>10</sub> TBC <sup>d</sup> (range)	Lysozyme (μg/ml of serum) <sup>e</sup> (range)
1	6/6 (3)	4.1 (3.8–4.4)	6.89	8.34 (7.61–9.18)	108.5 (106.7–111.3) <i>P</i> < 0.01
2	4/6 (0)	4.1 (1.6–7.3)	5.89	7.34 (2.90–10.12)	87.6 (72.2–101.5) <i>P</i> < 0.02
3	2/6 (1)	2.4 (1.5–4.9)	4.89	2.01 (ND <sup>f</sup> –10.03)	79.7 (62.3–108.2)
4	2/6 (1)	2.5 (1.4–6.3)	3.89	4.02 (ND <sup>f</sup> –10.04)	96.8 (87.6–116.0) <i>P</i> < 0.01
5	1/6 (1)	1.6 (1.5–1.8)	2.89	2.09 (ND <sup>f</sup> –3.83)	77.4 (67.3–85.1) <i>P</i> < 0.05
Control	0/4	1.9 (1.7–2.5)	PBS	ND <sup>f</sup>	65.6 (59.8–70.0)

<sup>a</sup> Rats were transtracheally inoculated with serial 10-fold dilutions of KP1 in 0.05 ml of sterile PBS.

<sup>b</sup> Surviving animals were sacrificed on day 6 post-inoculation, and lungs were removed aseptically and homogenized as described in the text. Animals were considered to be infected if they succumbed to their pneumonia or if their TBC was  $5 \times 10^4$  CFU or greater.

<sup>c</sup> KP1 was grown in TSB in mid-logarithmic phase (optical density at 550 nm) and washed three times in sterile PBS.

<sup>d</sup> As described in table 1, footnote b.

<sup>e</sup> Lysozyme assay performed by the procedure of Osserman and Lawlor (22), employing human urine lysozyme as the standard.

<sup>f</sup> ND, None detected at  $10^{-2}$  dilution of lung homogenate.

Table 4 shows the results obtained when various concentrations of KP1-O were inoculated transtracheally into the lungs of normal rats. All rats in the first three groups were infected by our criteria. In groups 4 and 5, three of the four rats in each group were infected. One rat in each group died, and serum lysozyme, as well as lung weight, was elevated. Finally, a dose of  $5.01 \times 10^1$  CFU or less of KP1-O did not result in an infection of any rats.

Table 5 shows the results obtained when various concentrations of KP1-T were inoculated transtracheally into the lungs of healthy rats. An initial inoculum of  $7.07 \times 10^7$  CFU of KP1-T

resulted in the death of three of the four rats in the first group. The one remaining rat effectively cleared this massive inoculum of KP1-T organisms placed in its lungs and showed no signs of pathology. Only one of the four rats in the second group succumbed to the infection, whereas the remaining three rats showed no signs of infection at the time of sacrifice. One of four rats in group 3 showed signs of infection, whereas none of the remaining rats in any of the other groups became infected.

Table 6 shows the results obtained when various concentrations of KP2-O were inoculated transtracheally into the lungs of normal rats. Doses of  $7.2 \times 10^7$  CFU did not result in a lobar pneumonia in three of the four animals in the first group. No rats in group 2 were considered infected, whereas one of the four rats in group 3 was infected, but all of the other rats in this group and in the ensuing groups (groups 4 to 6) had cleared *K. pneumoniae* from their lungs. Serum lysozyme was not significantly elevated in any group within either the KP1-T or the KP2-O study.

The ID<sub>50</sub> in rats for each of the four *K. pneumoniae* strains employed in these studies can be seen in Table 3, along with the LD<sub>50</sub> values for each strain in mice. These data show that KP1-O, which exhibited the largest capsule by India ink examination, was also the most virulent of these strains, both in the mouse and in the rat model. Its co-variant KP1-T was 4 or more log<sub>10</sub> units less virulent in both models. The KP1-T capsule was comparatively small (approximately 0.4 μm thick) under oil immersion. KP2-O possessed a capsule of similar thickness as that of KP1-T, and there seemed to be little difference in virulence between these

TABLE 3. LD<sub>50</sub> in mice and ID<sub>50</sub> in rats for various strains of *K. pneumoniae* serotypes 1 and 2

Organism	LD <sub>50</sub> (CFU) <sup>a</sup>	ID <sub>50</sub> (CFU) <sup>b</sup>
KP1 (mixed)	$1.92 \times 10^3$	$1.55 \times 10^5$
KP1-O	$4.99 \times 10^{1c}$	$3.41 \times 10^2$
KP1-T	$5.34 \times 10^{5c}$	$1.53 \times 10^7$
KP2 (mixed)	$4.29 \times 10^5$	ND <sup>d</sup>
KP2-O	$1.78 \times 10^5$	$>7.3 \times 10^7$
KP2-T	$>6.2 \times 10^7$	ND <sup>d</sup>

<sup>a</sup> Five groups of five mice each were inoculated intraperitoneally with serial 10-fold dilutions of the appropriate *K. pneumoniae* strain in 1.0 ml of sterile PBS and observed for a 72-h period. LD<sub>50</sub> values were calculated by the method of Reed and Muench (25) and represent at least two determinations for each organism.

<sup>b</sup> Rats were considered to be infected if they succumbed to their pneumonia or if the TBC was  $5 \times 10^4$  CFU or greater.

<sup>c</sup> The LD<sub>50</sub> values for KP1-O and KP1-T were shown to be significantly different (*P* < 0.025) by the two-tailed Student *t* test (28).

<sup>d</sup> ND, Not done.

TABLE 4. Establishment of a chronic lobar pneumonia in rats employing KP1-O

Group <sup>a</sup>	No. of animals infected/no. inoculated <sup>b</sup> (deaths)	Wt of lung (g) (range)	Log <sub>10</sub> initial inoculum (CFU) <sup>c</sup>	Log <sub>10</sub> TBC <sup>d</sup> (range)	Lysozyme (μg/ml of serum) <sup>e</sup> (range)
1	4/4 (3)	7.2	6.70	9.16	88.6
2	4/4 (1)	5.4 (3.8–6.3)	5.70	8.34 (6.64–9.22)	84.5 (79.0–92.1)
3	4/4 (1)	7.3 (6.7–8.2)	4.70	9.64 (9.46–9.92)	90.2 (76.4–105.7)
4	3/4 (1)	3.9 (2.0–4.4)	3.70	5.82 (ND <sup>f</sup> –8.78)	76.4 (55.5–86.9)
5	3/4 (1)	4.9 (2.2–8.6)	2.70	6.21 (ND <sup>f</sup> –10.22)	73.7 (59.1–83.3)
6	0/4 (0)	2.1 (1.9–2.4)	1.70	ND <sup>f</sup>	62.0 (61.8–63.5)
7	0/4 (0)	2.1 (1.7–2.4)	0.70	ND <sup>f</sup>	64.2 (60.9–66.2)
Control	0/4 (0)	1.9 (1.7–2.5)	PBS	ND <sup>f</sup>	65.6 (59.8–70.0)

<sup>a</sup> As described in Table 2, footnote a.<sup>b</sup> As described in Table 2, footnote b.<sup>c</sup> KP1-O was grown in defined medium described in the text to mid-logarithmic phase (optical density at 550 nm = 0.1) and washed three times in sterile PBS.<sup>d</sup> As described in Table 1, footnote c.<sup>e</sup> As described in Table 2, footnote e.<sup>f</sup> ND, None detected at 10<sup>-2</sup> dilution of lung homogenate.

organisms. KP2-T appeared to possess the smallest capsule and was the least virulent of these strains. A positive correlation of 0.98 was found to exist between the ID<sub>50</sub> values for the serotype 1 strains in the rat lung model and their respective LD<sub>50</sub> values in mouse lethality tests.

### DISCUSSION

Classically, the histological and pathological features of *Klebsiella* sp. pneumonia in humans include a massive, confluent lobar consolidation consisting primarily of polymorphonuclear leukocytes, a voluminous edema, and abscess formation with massive cavitation. Unfortunately, at least for the development of animal models, there exists a wide diversification of clinical manifestations of this disease process. Several classifications have been proposed which distin-

guish between an acute and chronic pneumonia pattern (11, 12, 16, 26), a primary versus a secondary (suprainfecting) pneumonia (12, 16, 26), and endogenous or epidemic sources of the organism (23). The progression of the disease and its prognosis are primarily related to age and predisposing variables (29). Since most *Klebsiella* sp. lobar pneumonias are seen in debilitated, middle-aged males (16, 23, 29), it is difficult to establish a model that closely approximates the human condition, especially when the large number of predisposing factors are considered. The rats used in the present study were healthy, young males more likely to effectively combat the experimental infection than the aged and debilitated human patient. This may explain the relatively low mortality and the greater chronicity of infection seen in this rat model. Neverthe-

TABLE 5. KP1-T in the rat lung model

Group <sup>a</sup>	No. of animals infected/no. inoculated <sup>b</sup> (deaths)	Wt of lung (g) (range)	Log <sub>10</sub> initial inoculum (CFU) <sup>c</sup>	Log <sub>10</sub> TBC <sup>d</sup> (range)	Lysozyme (μg/ml of serum) <sup>e</sup> (range)
1	3/4 (3)	1.9	7.85	ND <sup>f</sup>	69.9
2	1/4 (1)	2.2 (1.8–2.7)	6.85	1.20 (ND <sup>f</sup> –3.60)	65.5 (60.6–68.3)
3	1/4 (0)	2.3 (2.0–2.9)	5.85	3.01 (ND <sup>f</sup> –4.96)	64.7 (60.6–67.4)
4	0/4 (0)	2.4 (1.8–2.8)	4.85	ND <sup>f</sup>	65.5 (58.9–70.0)
5	0/4 (0)	2.0 (1.7–2.2)	3.85	0.84 (ND <sup>f</sup> –3.35)	59.2 (54.7–61.6)
6	0/4 (0)	1.9 (1.8–2.0)	2.85	ND <sup>f</sup>	58.2 (53.8–64.2)
Control	0/4 (0)	1.9 (1.7–2.5)	PBS	ND <sup>f</sup>	65.6 (59.8–70.0)

<sup>a</sup> Rats were inoculated transtracheally with serial 10-fold dilutions of viable KP1-T in 0.05 ml of sterile PBS.<sup>b</sup> As described in Table 2, footnote b.<sup>c</sup> KP1-T was grown in defined medium described in the text to mid-logarithmic phase (optical density at 550 nm = 0.2) and washed three times in PBS.<sup>d</sup> As described in Table 1, footnote c.<sup>e</sup> As described in Table 2, footnote e.<sup>f</sup> ND, None detected at 10<sup>-2</sup> dilution of lung homogenate.

TABLE 6. KP2-O in the rat lung model

Group <sup>a</sup>	No. of animals infected/no. inoculated <sup>b</sup>	Wt of lung (g) (range)	Log <sub>10</sub> initial inoculum <sup>c</sup>	Log <sub>10</sub> TBC <sup>d</sup> (range)	Lysozyme (μg/ml of serum) <sup>e</sup> (range)
1	1/4	2.6 (2.1–3.2)	7.86	3.91 (3.20–4.72)	66.1 (61.4–69.4)
2	0/4	2.0 (1.7–2.3)	6.86	3.02 (ND <sup>f</sup> –4.38)	55.9 (54.3–57.9)
3	1/4	2.0 (1.4–3.1)	5.86	3.17 (ND <sup>f</sup> –5.66)	64.7 (53.5–73.8)
4	0/4	2.1 (1.8–2.9)	4.86	1.56 (ND <sup>f</sup> –3.38)	66.3 (51.7–78.2)
5	0/4	1.6 (1.5–1.8)	3.86	1.76 (ND <sup>f</sup> –3.88)	64.5 (61.6–68.4)
6	0/4	1.8 (1.4–2.1)	2.86	2.13 (ND <sup>f</sup> –4.63)	66.7 (62.4–69.9)
Control	0/4	1.9 (1.7–2.5)	PBS	Nd <sup>f</sup>	65.6 (59.8–70.0)

<sup>a</sup> Rats were inoculated transtracheally with serial 10-fold dilutions of viable KP2-O in 0.05 ml of sterile PBS.

<sup>b</sup> As described in Table 2, footnote b. No lethality was seen with this model.

<sup>c</sup> KP2-O was grown in defined medium as described in text to mid-logarithmic phase (optical density at 550 nm = 0.2) and washed three times in sterile PBS.

<sup>d</sup> As described in Table 2, footnote d.

<sup>e</sup> As described in Table 2, footnote e.

<sup>f</sup> ND, None detected at 10<sup>-2</sup> dilution of lung homogenate.

less, in the present study, we were able to establish a rat model which displayed the classical symptoms for *K. pneumoniae* pneumonia found in humans.

Berendt et al. (3) produced a bronchopneumonia in rats with intranasal inoculations of  $5 \times 10^6$  *K. pneumoniae* (strain A-D). The authors eventually abandoned their rat model, concluding that the squirrel monkey provided a more satisfactory experimental model for lobar pneumonia. This nonhuman primate model allows measurements of clinical signs that a rodent model does not afford, such as fever, respiratory rate, and throat cultures (2). However, the squirrel monkey pneumonia pattern mimics only the acute form of the disease. The rapidity with which death occurred and the low frequency of abscess formation severely restricted the utility of this model. Moreover, in their discussion (2), the authors noted the economic, practical, and statistical advantages of using a rodent model to study this type of infectious process.

Sale and Wood (27) reported the production of a lobar pneumonia in rats. They described a highly acute infection, with the majority of their rats succumbing to their pneumonia by day 3 postexposure. Again, their model simulated, at best, the acute pattern of *Klebsiella* sp. pneumonia in humans. The high mortality encountered by these researchers was most likely due to the administration of mucin into the lungs, a procedure that other authors felt could have "profound effects" on the experimental animal (2).

In the present study we were able to produce a chronic lobar pneumonia in rats without the aid of mucin (Table 1). This model allows for the colonization and infection of the rat lung by *K. pneumoniae* for at least 28 days. Previous attempts at establishing an experimental paradigm for *Klebsiella* sp. respiratory infections have disregarded the infectivity of the bacterium. In

this report, two serotypes and two variants within these serotypes were examined for their ability to produce a chronic lobar pneumonia in rats, and a comparison was made with their virulence in mouse lethality tests. The results show how important it is to determine the pathogenic nature of the organism before model construction is possible (Table 3).

Establishing the threshold of infection in these rats at a TBC of  $5 \times 10^4$  was based on several meaningful observations. First, it was at this approximate inoculation titer when morphological changes in the lungs were seen. Berendt et al. (3) reported that lysozyme levels did not become elevated until the number of bacteria in the lungs reached 4 to 5 logs. Serum lysozyme is a convenient assay for determining the extent of infection because it reflects the appearance, frequency, and severity of pyogranulomatous lesions (4). Second, with only a few exceptions, the present study showed an all or none response to the *K. pneumoniae* challenge, with rat lungs containing either well over this  $5 \times 10^4$  CFU threshold or well below it. Third, changes in lung weight also supported a TBC of  $5 \times 10^4$  CFU as the threshold of infection. None of the rats with a lung content of under  $5 \times 10^4$  bacteria had any marked elevation in lung weight.

It has been shown in this report that the particular *K. pneumoniae* strain utilized has a strong bearing on the nature and extent of the disease produced. Furthermore, variants within a single population of *K. pneumoniae* show marked differences in pathogenicity. These differences are believed to be associated with capsular polysaccharide (CPS) production. It is well established that the presence of a capsule is essential for the virulence of *K. pneumoniae* (10, 17), and these studies suggest that the antiphagocytic properties of the capsules increase as the cell-associated capsular material increases in

volume. The results of the present study support such a relationship. The capsular volume of KP1-O was much larger than that of KP1-T, and virulence correlated positively within this serotype. The same held true for the KP2 strain, whose variants differed only very slightly in capsule size. There also appears to be no correlation between serotype and virulence (9, 21), suggesting that many, if not all, *Klebsiella* serotypes may be capable of producing copious amounts of CPS.

Though our findings lend support to the relationship between capsule size and virulence, it by no means rules out other possible explanations for the marked differences in pathogenicity seen among *K. pneumoniae* strains. One of the more attractive theories to explain the differences in pathogenicity of *K. pneumoniae* proposes that the amount of soluble CPS produced and exuded into the medium by a given strain correlates with virulence. This has already been shown to be the case for the type III group B streptococci (7). Circulating cell-free CPS in the blood of a patient infected with *K. pneumoniae* could conceivably neutralize any previously or newly synthesized antibodies before opsonization occurred (24). In addition, Batshon et al. (1) induced immunological paralysis in mice with the CPS isolated from KP2, and this same phenomenon has been previously shown with the CPS from *Streptococcus pneumoniae* (20). Pollack (24) demonstrated that the presence of detectable CPS in the serum of patients infected with *K. pneumoniae* appeared to correlate with the severity of infection, with persistence of active foci, and with a poorer prognosis than in those patients who had no detectable circulating antigen. This phenomenon was also observed in an earlier study with *S. pneumoniae* (5), as well as in the case of group B meningococemia (14). Furthermore, it has been shown that the in vitro presence of minute quantities of CPS produced by *K. pneumoniae* inhibits the maturation and functional capacity of macrophages (30, 31). In one of the studies already mentioned (10), it was shown that there was a direct correlation between capsular size and the concentration of soluble CPS produced by four different strains of *K. pneumoniae*. Thus, the direct relationship between capsule size and virulence may be due to the fact that extracellular, soluble CPS increases as capsular CPS increases. There now appears to be a great deal of mounting evidence to support the role of soluble CPS in the pathogenicity of encapsulated bacteria.

We believe that both the cell-associated capsule and the cell-free slime may play a role in *Klebsiella* sp. infections. Although the presence of a capsule serves to impede phagocytosis, the release of capsular material may inhibit the

activation of other host defenses. Release of the soluble CPS may also accentuate the antiphagocytic character of a given strain, especially if it is synthesized at a rapid rate (10). It is quite possible that the gel-like property of *Klebsiella* sp. CPS in solution may act to sequester the growing bacteria from nonspecific host factors. Further examination of these phenomena would appear to be warranted. The chronic lobar pneumonia established in this study may allow for a more meaningful in vivo study of the above-mentioned hypotheses and a closer examination of the pathogenicity of *K. pneumoniae* in lower respiratory tract infections.

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