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Urinary Virulence of *Proteus mirabilis* in Two Experimental Mouse Models

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Two experimental mouse models were tested for their suitability in measuring urinary virulence of *Proteus mirabilis*. In the first model, the kidney-infecting dose and lethal dose were measured. In the second model, the kinetics of the numbers of bacteria in the kidneys and other organs of the mouse were monitored for 13 h after injection.

Proteus mirabilis is an important urinary pathogen (2, 9), especially in nosocomial infections (13) and in infections in young male children (6, 9, 10). To elucidate further the role of suggested virulence factors such as urease (4, 5, 7, 11), sensitivity to the bactericidal effect of serum (3), and the possession of pili (12) and flagellae (8) and to see whether differences in virulence are correlated to the course of human urinary disease, we decided to examine two experimental mouse models for their suitability in measuring the virulence of *P. mirabilis* strains.

The mice used in these experiments were albino Swiss mice (Swiss Random, TN0, Zeist, The Netherlands) and weighed approximately 25 g.

In the first model, five groups of six male mice (6 weeks old) were injected intravenously with twofold dilutions of a bacterial suspension of late-log-phase cells prepared by the method of Van den Bosch et al. (14), using saline instead of Ringer solution as a suspending medium. After 10 days, the surviving mice were killed. All mice surviving for more than 4 days were examined macroscopically for kidney infection. The 50% lethal dose (LD_{50}) and the 50% kidney-infecting dose (KID_{50}) were calculated by the method of Spearmann and Kärber (1).

The model of Van den Bosch et al. (14) was used as a second model with some minor alterations. Ten female mice (8 weeks old) were injected intravenously with 2.0×10^8 bacteria. At 0.25, 2, 4.5, 7.5, and 13 h after injection, two random mice were killed, and the numbers of bacteria in the kidneys, liver, spleen, and blood were determined. The kidney and spleen values are based on two independent experiments. For the liver and blood counts, no duplicate experiments were carried out.

Thirty-four strains were tested in both models. The results of the LD_{50} and KID_{50} experiments are shown in Table 1. The strains were assigned to group I with a KID_{50} of over 2×10^8 or to group II with a KID_{50} of under 2×10^8. For strains with a KID_{50} close to the LD_{50}, it was only possible to calculate the KID_{50} by assuming that the mice dying within a few days after injection would have developed kidney infections in the same ratio as for the surviving mice. The consequence is that in these cases, the calculated KID_{50} value should be considered as a rather rough estimate. Bacteriological examination of more than 100 kidneys macroscopically proved that macroscopic examination for the presence of kidney infections was justified.

The results of the kinetic experiments based on mean values for 11 strains out of each group are shown in Fig. 1 to 4. The strains from the two groups behaved differently in this model. Strains from group I showed low numbers in the kidneys throughout the experiment. Strains from group II produced higher initial numbers in the kidneys and, after an initial decrease in the first hours, showed a steady increase in numbers. The values for the liver, spleen, and blood showed a faster decrease for group I strains compared with group II strains. Statistical analysis with the Mann-Whitney test showed that the mean numbers of bacteria in different organs at all times were significantly higher for group II strains compared with group I strains ($P < 0.05$), with the exception of the liver counts at 0.25 and 2 h after injection.

The behavior patterns in the kidneys were dose dependent. After the injection dose was raised to more than the KID_{50} value, group I strains showed the same behavior as group II strains in the kidneys, and the opposite phenomenon was seen for group II strains after the injection dose was lowered to less than the KID_{50} value.

Of a further 12 strains tested in both experiments, only 1 did not conform to the patterns we

TABLE 1. LD_{50} and KID_{50} values for the two groups of *P. mirabilis* strains

Group I				Group II			
Strain	LD_{50}	KID_{50}	Ratio[a]	Strain	LD_{50}	KID_{50}	Ratio[a]
AM9	3.1×10^8	2.5×10^8	1.3	AM12	6.2×10^8	1.2×10^8	5.2
AM35	7.9×10^8	2.8×10^8	2.8	AM13	3.8×10^8	7.6×10^7	5.0
AM39	9.8×10^8	3.5×10^8	2.8	AM41	3.7×10^8	9.1×10^7	4.1
AM40	7.1×10^8	3.1×10^8	2.3	AM48	8.2×10^8	4.6×10^7	17.8
AM45	7.1×10^8	4.5×10^8	1.6	AM49	2.7×10^8	4.3×10^7	6.3
AM46	8.5×10^8	3.0×10^8	2.8	AM52	3.0×10^8	6.7×10^7	4.5
AM47	1.1×10^9	3.6×10^8	3.1	AM53	1.6×10^8	9.0×10^7	1.8
AM51	9.1×10^8	4.5×10^8	2.0	AM57	3.9×10^8	1.6×10^8	2.4
AM54	2.1×10^8	2.1×10^8	1.0	AM60	7.6×10^8	9.7×10^7	7.8
AM75g	1.7×10^9	1.1×10^9	1.5	AM63	5.9×10^8	4.7×10^7	12.6
AF1	8.9×10^8	7.1×10^8	1.3	AM73	3.5×10^8	1.4×10^8	2.5
AM8	5.4×10^8	4.3×10^8	1.3	AM28	1.4×10^8	4.0×10^7	3.5
AM18	5.8×10^8	3.3×10^8	1.8	AM38	1.6×10^8	7.8×10^7	2.1
AM21	1.2×10^9	2.0×10^8	6.0	AM42	5.8×10^8	1.3×10^8	4.5
AM22	3.5×10^8	2.0×10^8	1.8	AM43	1.1×10^8	9.5×10^7	1.2
AM44	7.1×10^8	6.3×10^8	1.1	AM17	2.1×10^8	1.8×10^8	2.1
AM61	5.6×10^8	2.9×10^9	1.9	AM7	1.0×10^9	1.6×10^8	6.25
Mean	6.9×10^8	3.6×10^8	1.9		3.5×10^8	8.8×10^7	4.0

[a] LD_{50}/KID_{50}.

have described. The typical patterns based on the mean of 11 strains for each group were also displayed by each individual strain with some variation. Similar patterns were described for *Escherichia coli* strains (14). Group II strains of *E. coli* were considered by Van den Bosch et al. (14) as specially nephropathogenic. Whether the same is true for group II strains of *P. mirabilis* is difficult to answer. In favor of this theory are the higher lodgment in the kidneys of group II strains after injection, and the growth of these strains in the kidneys finally resulted in kidney infections and a higher LD_{50}/KID_{50} ratio for these strains. It cannot be excluded, however, that these phenomena may reflect a higher general virulence because of an increased resistance to such general defense mechanisms as phagocytosis. Our experimental data allow no definite conclusions. For virulence measurements of *P. mirabilis*, we prefer the LD_{50}/KID_{50} model to the kinetic model. Whereas the latter only permits the division of the strains in two virulence groups based on an arbitrarily chosen injection dose, the former model makes possible a rather

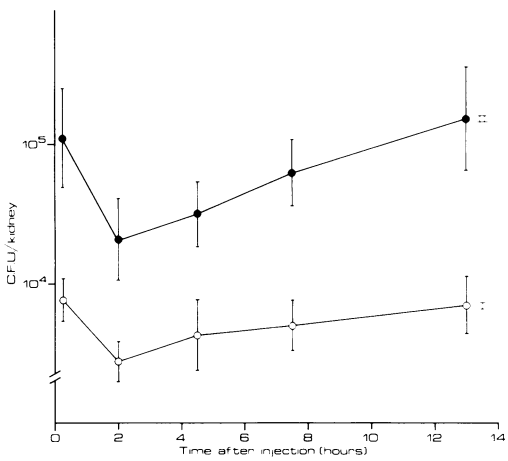

FIG. 1. Kinetics of the viable counts in the kidneys for group I and II strains. Mean of 11 strains per group. CFU, Colony-forming units.

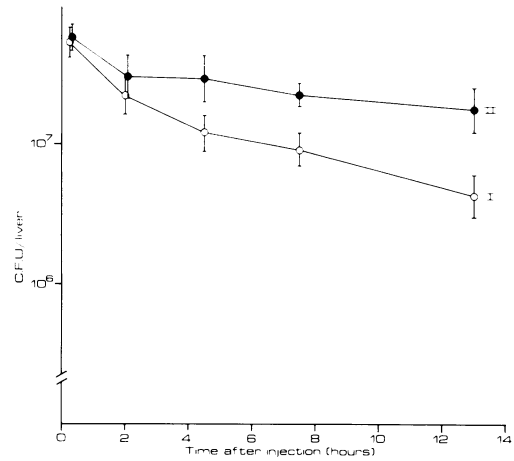

FIG. 2. Kinetics of the viable counts in the liver for group I and II strains. Mean of 11 strains per group. CFU, Colony-forming units.

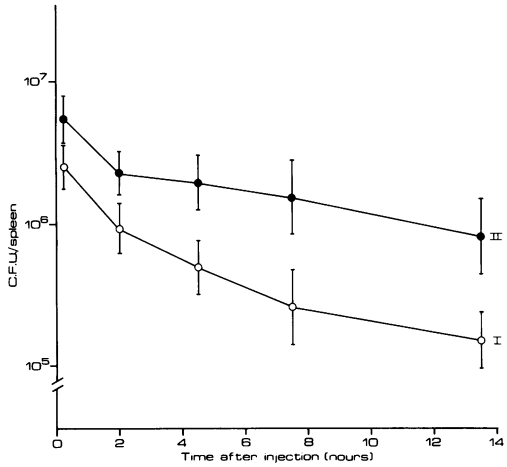

FIG. 3. Kinetics of the viable counts in the spleen for group I and II strains. Mean of 11 strains per group. CFU, Colony-forming units.

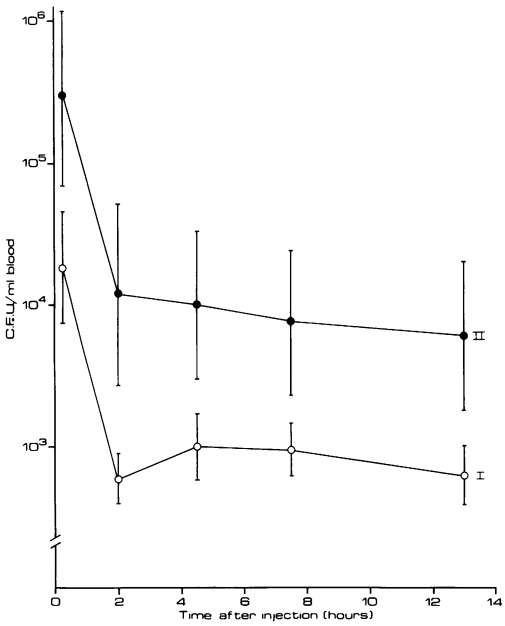

FIG. 4. Kinetics of the viable counts in the blood for group I and II strains. Mean of 11 strains per group. CFU, Colony-forming units.

accurate judgement of two virulence parameters, the LD_{50} and the KID_{50}. It will be interesting to examine whether differences in virulence found in this model can be correlated with differences in origin of the strains or with other properties. Such studies are in progress.

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