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Virulence in Rats of Gentamicin-Carbenicillin-Resistant *Pseudomonas*

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Strains of *Pseudomonas aeruginosa*, with resistance to gentamicin and carbenicillin which is R factor mediated, showed no alteration in virulence as tested by intraperitoneal injection of rats.

Recent reports in both the clinical and microbiological literature have described *Pseudomonas aeruginosa* strains as resistant to both carbenicillin and gentamicin (2, 4, 5, 8). Organisms with a similar resistance pattern have been isolated with increasing frequency from 1971 through 1974 at the Cincinnati Veterans Administration Hospital. Simultaneously, resistance transfer factors (R) responsible for the development of resistance in a majority of these strains were demonstrated (6). Our clinical observations suggested that the resistant *Pseudomonas* were less virulent than we anticipated. Similar findings were described by Green et al. (4). To determine whether or not the presence of the R factor resulted in decreased virulence of the *Pseudomonas*, in vivo studies were conducted in which rats were challenged intraperitoneally with the organism, with and without R factor.

MIC determinations. The minimal inhibitory concentrations (MIC) of gentamicin and carbenicillin for the *Pseudomonas* organisms used in these experiments were determined by a standard twofold serial tube dilution method using a total volume of 1 ml as previously reported (7).

Animal studies. In two experiments, pathogen-free Sprague-Dawley female rats weighing 200 g were challenged intraperitoneally with *Pseudomonas* according to the method previously described by Andriole (1).

In the initial experiment a clinical isolate with a gentamicin-susceptible ($G^S C^S$) pattern of antibiotic resistance was chosen. This strain was then converted to $G^R C^R$ by a laboratory mating with a donor strain which carried a $G^R C^R$ plasmid representative of the local R^+ population (6). The gentamicin MIC of the $G^S C^S$ strain was 1.56 μg/ml; the carbenicillin MIC was 125 μg/ml. For the $G^R C^R$ derivative, the gentamicin MIC was greater than 25 μg/ml and the carbenicillin MIC was greater than 500 μg/ml. A total of 60 animals were challenged; six served as control and received only sterile media. Two groups of 12 were inoculated with 10^8 colony-forming units (CFU) of the $G^S C^S$ or $G^R C^R$ variant, and two groups of 15 were challenged with 2×10^7 CFU of the two variants.

The second experiment compared the virulence of the original $G^R C^R$ isolate (R+) and a spontaneously reverted antibiotic-susceptible variant (R−). This susceptible variant had a gentamicin MIC of 0.78 μg/ml and a carbenicillin MIC of 125 μg/ml. Fifty animals were studied. Two control animals were inoculated with sterile media; six animals received 10^8 CFU, 12 received 5×10^7 CFU, and six received 1×10^7 CFU of either variant.

Survival of inoculated animals was noted at 6, 18, 24, 48, and 72 h.

In the initial experiment, rats were inoculated intraperitoneally either with a clinical isolate which was $G^S C^S$ or with the $G^R C^R$ variant which was isolated after mating to a $G^R C^R$ strain. All controls survived (Fig. 1). Seventeen percent of the animals inoculated with 10^8 $G^R C^R$ survived at 18, 24, and 72 h; none of the animals challenged with 10^8 $G^S C^S$ survived 24 h. The survival curves of the two groups of animals challenged with 2×10^7 were identical. Rats inoculated with the low number of CFU showed a survival rate twice that of rats receiving 10^8 CFU.

A second series of intraperitoneal challenges was carried out with a clinical isolate R+ strain and its spontaneously derived R− derivative. Both control animals survived after administration of sterile media (Fig. 2). At 18 h, 17% of the animals inoculated with 10^8 CFU of the R− variant and none receiving the susceptible organisms survived. By using 5×10^7 CFU, the 72-h survival after inoculation with the R+ was 25%, whereas 42% of the rats given an identical

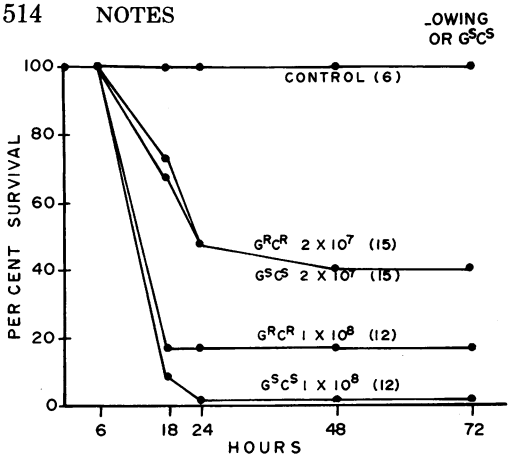

FIG. 1. *The percentage of survival over 72 h of animals challenged intraperitoneally with 2 × 10^7 and 10^8 G^RC^R and G^SC^S Pseudomonas. Numbers in parentheses indicate the number of rats in each group.*

inoculum of the susceptible variant survived. At the lowest inoculum, 10^7 CFU, survival in rats given the R+ variant was 33%, and in animals receiving the susceptible organisms, 17%.

The appearance of *P. aeruginosa* resistant to the clinically most efficacious anti-pseudomonas agents, gentamicin and carbenicillin, might have been expected to yield serious consequences, particularly in patient populations prone to *Pseudomonas* colonization. Nevertheless, the experience at the Baltimore Cancer Research Center (4) in patients with severely compromised defense was just the opposite.

From studies of isolates (6), it is likely that most of the antibiotic-resistant *Pseudomonas* recovered in this hospital during the period 1971 through 1974 have contained G^RC^R resistance plasmids. It was postulated that the clinical observations of apparent decreased virulence in the resistance might be related to effects of the plasmid itself rather than to the specific antibiotic resistance traits. In the present study, however, the survival of rats inoculated intraperitoneally with a G^RC^R variant did not show increased survival, relative to that of rats receiving the G^SC^S organism. In addition, no consistent differences were observed in a similar experiment which used a clinically isolated R+ strain and its spontaneously derived R− variant. Virulence of *P. aeruginosa* for the rat appears to be independent of the presence or absence of the plasmid mediating specific antibiotic resistance patterns. The clinical impression of the low virulence of the G^RC^R remains unexplained.

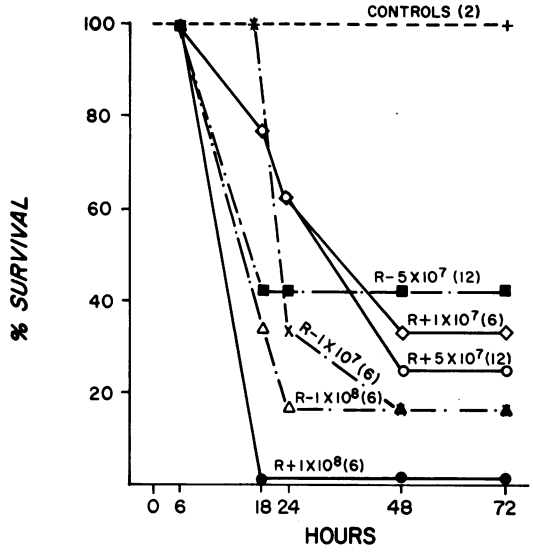

FIG. 2. *The percentage of survival over 72 h of animals challenged intraperitoneally with 10^7, 5 × 10^7, and 10^8 R+ and R− Pseudomonas. Numbers in parentheses indicate number of rats in each group.*

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