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Impaired Virulence and Fitness of a Colistin-Resistant Clinical Isolate of *Acinetobacter baumannii* in a Rat Model of Pneumonia

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We compared the fitness and lung pathogenicity of two isogenic clinical isolates of *Acinetobacter baumannii*, one resistant (ABCR) and the other susceptible (ABCS) to colistin. *In vitro*, ABCR exhibited slower growth kinetics than ABCS. In a rat model of pneumonia, ABCR was associated with less pronounced signs of infection (lung bacterial count, systemic dissemination, and lung damage) and a better outcome (ABCR and ABCS mortality rates, 20 and 50%, respectively [$P = 0.03$]).

The emergence of colistin-resistant strains of *Acinetobacter baumannii* is perceived as a formidable threat in clinical settings. Recent contradictory clinical observations and experimental data have questioned the virulence of such strains (1, 2, 3). Experimental models of surgical infections have previously assessed the virulence of *A. baumannii* with *in vitro*-induced colistin resistance (4), but a specific model of pneumonia with parental clinical isolates is required to focus on lung pathogenicity.

We compared the fitness and virulence, *in vitro* and *in vivo*, of *A. baumannii* according to its sensitivity to colistin in a rat model of acute pneumonia using two isogenic clinical strains.

Two strains of *A. baumannii* were successively isolated from the respiratory tract of a patient with ventilator-associated pneumonia. The first was colistin susceptible (ABCS), and the second was colistin resistant (ABCR). Whole-genome sequencing confirmed that they were parental strains, with ABCR differing from ABCS by a mutation in the *pmrA* and *rpoB* genes and by the loss of a prophage (5, 6).

The ABCS strain was obtained from bronchoalveolar lavage fluid (colistin MIC = 0.064 mg/liter) (5). The ABCR strain was isolated from tracheal aspirates after the patient had received intravenous colistin (colistin MIC = 32 mg/liter) (5). Apart from the colistin and rifampin susceptibility of ABCS, both strains were resistant to all of the antibiotics tested, including cefepime and sulbactam.

The 24-h growth curves of ABCR, ABCS, and reference strain AYE (7) showed a reduced slope in the exponential phase for ABCR versus ABCS (0.14 ± 0.0038 versus 0.18 ± 0.0053 [$P = 0.01$]) and versus AYE (0.14 ± 0.0038 versus 0.23 ± 0.0066 [$P = 0.003$]), indicating slower growth of ABCR bacteria.

Both the ABCR and ABCS strains were tested for virulence in an animal model of acute pneumonia. Adult Sprague-Dawley male pathogen-free rats (weighing 300 to 350 g) were used for *in vivo* experiments after approval was obtained from the departmental ethics committee (study agreement 58-08112012).

Sixty animals received 250 μl of phosphate-buffered saline (PBS) containing 10^9 CFU/ml of ABCR ($n = 30$) or ABCS ($n = 30$) and 250 μl of porcine mucin diluted to 10% via the tracheal route. Ten controls received only 250 μl of PBS and 250 μl of porcine mucin. The follow-up period lasted 48 h with daily body weight recording. After death, blood and spleen samples were cultured to assess bacteremia and the right lung was cultured. A histological severity score (HSS; minimum, 0; maximum, 4) was calculated on the basis of the number of bronchopneumonia lesions present in the left lung (8).

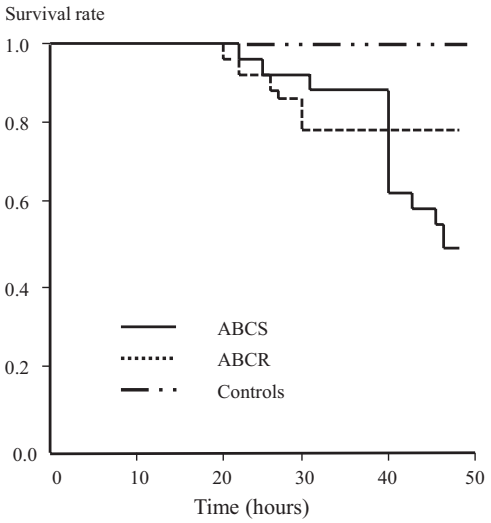

FIG 1 Forty-eight-hour survival curves of animals infected with the same inoculum of ABCS or ABCR compared to that of control (uninfected) animals. The log rank of the Kaplan-Meier curve was $P = 0.04$ between the ABCR and ABCS curves.

The data were expressed as the mean ± standard deviation or the median and interquartile range according to the distribution of the data. The difference in bacterial lung counts was analyzed by the Mann-Whitney test. Data analysis was performed with SPSS for Windows, version 12.0 (SPSS, Chicago, IL). $P \leq 0.05$ was considered statistically significant.

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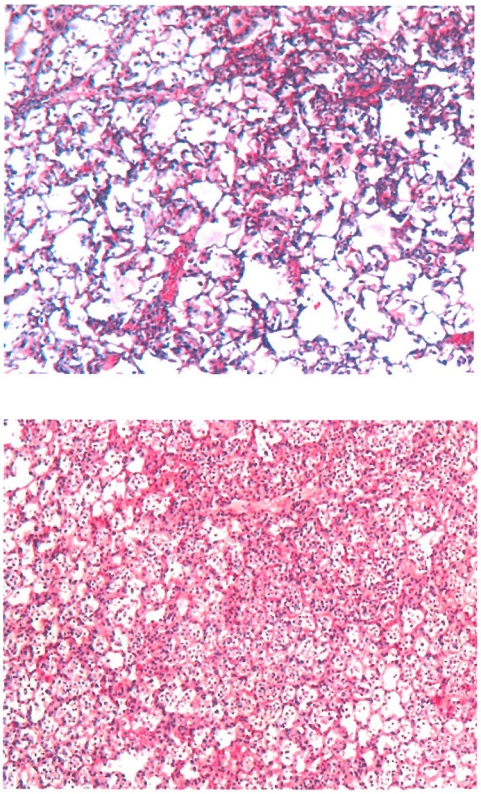

FIG 2 Pathological mapping of lung tissue samples representative of the ABCR and ABCS groups. Giemsa staining and a magnification of ×50 were used. ABCR lung tissue showed fewer confluent lesions of bronchopneumonia with areas of preserved lung architecture, in contrast to ABCS lung tissue.

The results showed that 6 (20%) of the 30 rats infected with ABCR died spontaneously during the first 48 h, compared with 15 (50%) of 30 in the ABCS group ($P = 0.03$). None of the 10 control animals died (Fig. 1).

The decrease in body weight was less pronounced in the ABCR group than in the ABCS group, with end follow-up values of 295 ± 55 g for ABCR and 263 ± 39 g for ABCS ($P = 0.029$).

The weight of the right lung indexed to body weight was lower in the ABCR group than in the ABCS group (6 [5.5 to 7.2] versus 9.2 [6.5 to 13.8] g/kg, respectively [$P < 0.001$]), attesting to less intense edema and lung injury. The indexed lung weight in the control group was 2.5 [2 to 3.2] g/kg.

The lung bacterial count was significantly lower in the ABCR group than in the ABCS group (1.4×10^6 [5.3×10^5 to 1.1×10^7] versus 1.6×10^9 [8.5×10^6 to 2.6×10^{10}] CFU/g of lung, respectively [$P = 0.011$]).

A lower percentage of the animals in the ABCR group showed bacteremia (15% versus 63% of those in the ABCS group [$P < 0.001$]).

The HSS was significantly lower in ABCR rats than in ABCS rats (2.5 ± 0.4 versus 3.8 ± 0.2, respectively [$P = 0.02$]). In the ABCR group, some areas of normal lung parenchyma could be observed and no abscesses were detected, in contrast to the ABCS group (Fig. 2).

In the present work, we developed a reproducible and reliable rat model of acute pneumonia with strains that emerged from environmental selection and showed that the colistin-resistant strain of *A. baumannii* had reduced growth kinetics and was less virulent than the colistin-susceptible one.

Mutation-induced changes in the expression of membrane proteins, cytoplasmic activation of signal factors, and metabolic enzymes have been suggested to be responsible for a reduction in virulence in colistin-resistant *A. baumannii* (9). A reduction of biofilm-forming ability related to the acquisition of colistin resistance by *A. baumannii* (10) has also been suggested to explain the loss of virulence.

Mechanisms of *A. baumannii* colistin resistance were recently reviewed in detail (1) and may involve (i) total loss of lipopolysaccharide and (ii) PmrAB two-component system-regulated lipid A modification. In our ABCR strain, resistance to colistin was associated with mutations in the PmrA (E8D)-encoding gene (5), arguing that the reduced virulence we report here was strongly associated with the colistin resistance phenotype of the ABCR strain. Differences between the two strains also included the loss of a prophage in the ABCR strain compared with the ABCS strain (5), which may have contributed to the decrease in *in vivo* virulence.

Our study demonstrated the reduced virulence and lung pathogenicity of the ABCR strain and is in agreement with the clinical outcome of the patient it came from (2). It would, however, be premature to extend our findings to other clinical settings or strains.

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