Increased Susceptibility to Colistin in Hypermutable *Pseudomonas aeruginosa* Strains from Chronic Respiratory Infections

A common feature of *Pseudomonas aeruginosa* chronic respiratory infections (CRI), including those occurring in patients suffering from cystic fibrosis, bronchiectasis, or chronic obstructive pulmonary disease, is a very high prevalence (30 to 60%) of hypermutable (or mutator) strains deficient in the DNA mismatch repair (MMR) system, in contrast to what is observed in acute processes (<1%) (1, 4, 10, 13, 14). The presence of hypermutable strains has been found to be linked to the high antibiotic resistance rates of *P. aeruginosa* clinical isolates recovered from patients with CRI (1, 10, 13), and in vitro and in vivo experiments have shown that hypermutation dramatically speeds up resistance development during exposure to antimicrobial agents (15, 16).

Colistin has reemerged in the last decade as a relevant therapeutic option for the treatment of infections caused by multidrug-resistant gram-negative bacteria (8). The objective of this work was to assess the activity of this antibiotic against hypermutable *P. aeruginosa* strains isolated from patients with CRI. Colistin susceptibility was determined by the Etest method, recently shown to be reliable for this purpose (3), in a collection of 97 *P. aeruginosa* isolates characterized in two previous studies (9, 10). This collection included one to four isolates recovered between 2003 and 2004 from each of 51 patients suffering from cystic fibrosis (n = 21), bronchiectasis (n = 22), or chronic obstructive pulmonary disease (n = 8). Each patient was shown to be chronically infected by a different *P. aeruginosa* clone, and of the 97 isolates, 43 were found to be hypermutable (most of them defective in the MMR gene *mutS*); a marked association between hypermutation and multidrug resistance was also documented when susceptibility to the commonly used antipseudomonal agents ceftazidime, imipenem, meropenem, ciprofloxacin, and tobramycin was analyzed in this collection (7, 8). Finally, a collection of 50 *P. aeruginosa* clinical isolates recovered from 50 patients admitted to our intensive care unit (ICU) between 2002 and 2003 was also tested for comparative purposes. None of these isolates was hypermutable, and each of them belonged to a different clonal type, as documented in a previous study (4). *P. aeruginosa* strain ATCC 27853 was used for quality control, yielding an MIC of 1 µg/ml, which falls within the acceptable range defined by the Clinical and Laboratory Standards Institute.

The distribution of the colistin MICs obtained for the hypermutable and nonhypermutable isolates from patients with CRI and the nonhypermutable isolates from ICU patients is shown in Fig. 1. In contrast to what was previously documented for other antimicrobial agents, MICs tended to be highest for ICU isolates (geometric mean MIC, 2.0 µg/ml) and lowest for hypermutable isolates from patients with CRI (0.48 µg/ml). Nonhypermutable isolates from patients with CRI showed an intermediate distribution of MICs (geometric mean MIC, 0.92 µg/ml). For only one of the strains (from the ICU isolate group) did the MICs surpass the currently recommended susceptibility breakpoint (≥4 µg/ml) (6). Statistical analysis (Mann-Whitney U test) of the distribution of the MICs yielded significant differences (*P* < 0.001) when hypermutable and nonhypermutable isolates from patients with CRI were compared; statistically significant differences (*P* < 0.001) were also obtained when nonhypermutable isolates from ICU and CRI patients were analyzed. Increased colistin susceptibility of the

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**FIG. 1.** Distribution of the MICs of colistin for hypermutable *P. aeruginosa* strains isolated from patients with CRI (n = 43), nonhypermutable *P. aeruginosa* strains isolated from patients with CRI (n = 54), and nonhypermutable *P. aeruginosa* strains isolated from ICU patients (n = 50).
hypermutable strains was not likely a direct consequence of the inactivation of the MMR system since the MICs for reference strain PAO1 and its mutS-deficient derivative PAOΔmutS (15) were found to be essentially identical (2 μg/ml).

It is noteworthy that almost none of the patients had ever been treated with colistin (which was only seldom used in our hospital until very recently), and therefore the documented cases often end up showing reduced in vitro and in vivo fitness, probably due to the unavoidable accumulation of deleterious mutations for secondary environments (7, 11, 12). Whether the observed colistin-hypersusceptibility of natural hypermutable strains remains to be elucidated. Although results from this work would certainly argue in favor of the use of colistin for attempting to counterselect hypermutable strains in CRI, they should be taken cautiously, since development of resistance to this agent under selective pressure is not unexpected (6), although in vitro experiments have suggested that it may occur to a lesser extent compared with other antipseudomonal agents (15).

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REFERENCES


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