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Decreased Susceptibility to Polymyxin B during Treatment for Carbapenem-Resistant *Klebsiella pneumoniae* Infection▽

In the United States, the major mechanism of carbapenem resistance among *Enterobacteriaceae* is the production of *Klebsiella pneumoniae* carbapenemase (KPC) (2). Polymyxins and tigecycline are the antimicrobials most often used for treatment of infections caused by carbapenem-resistant *Enterobacteriaceae* (4, 11). Polymyxin B, discovered more than 60 years ago, has been reintroduced as a valuable therapeutic agent with efficacy against multidrug-resistant gram-negative bacteria due to a shortage of new antimicrobials with activities against these organisms. While polymyxin B resistance has been observed in clinical isolates of carbapenem-resistant *K. pneumoniae* (CRKP), reports of developing resistance in vivo during treatment with polymyxin B are limited (1, 8).

We studied patients with CRKP infections who persistently had positive cultures despite ≥3 days of treatment with either polymyxin B alone or polymyxin B and tigecycline. The patients were hospitalized between July 2004 and June 2006 at the Mount Sinai Hospital (New York, NY). The history of antibiotic therapy and the duration of treatment were obtained from patients' medical records and hospital pharmacy records. Carbapenem resistance in *K. pneumoniae* isolates was initially detected by using an automated broth microdilution system (MicroScan; Dade Behring, Deerfield, IL), followed by confirmation with the disk diffusion assay and Etest (AB Biodisk, Solna, Sweden). The presence of the KPC gene in each isolate was tested by PCR analysis using primers and conditions described elsewhere (12). The MICs for polymyxin B and tigecycline were determined by Etest.

Of the 16 patients included in this study, 12 received polymyxin B (polymyxin B sulfate) alone for a mean duration of 12.6 days (range, 3 to 25 days) and 4 received both polymyxin B and tigecycline for a mean duration of 9.3 days (range, 4 to 23 days) and 9 days (range, 3 to 16 days), respectively. Polymyxin B and tigecycline were given as a combination therapy for a mean duration of 6.25 days (range, 3 to 15 days) for those who received both agents. All patients received a therapeutic dose of polymyxin B, which was renally adjusted if needed. The initial CRKP isolates were recovered from blood (12 patients), peritoneal fluid (2 patients), sputum (1 patient), and cerebrospinal fluid (1 patient) samples. All of the subsequent CRKP isolates were recovered from blood samples. The KPC gene was present in all initial and subsequent isolates as confirmed by PCR analysis. The mean time interval between the recovery of initial and subsequent isolates was 16.6 days (range, 4 to 55 days) for those who received polymyxin B alone and 15.5 days (range, 4 to 42 days) for those who received both polymyxin B and tigecycline. Significant increases in the polymyxin B MIC were observed in the subsequent isolates recovered from 3 of 12 patients (25%) treated with only polymyxin B: 1.5 μg/ml to 32 μg/ml, 0.75 μg/ml to 12 μg/ml, and 0.75 μg/ml to 1024 μg/ml, respectively (Table 1). The mean durations of treatment with polymyxin B for these three patients were not different from those for the other nine patients whose subsequent isolates did not have an increased MIC (mean, 15.7 days versus 10.8 days; $P = 0.46$). During the study period, more than 90% of CRKP clinical isolates at our institution demonstrated in vitro susceptibilities to polymyxin B. None of the subsequent isolates from patients who received both polymyxin B and tigecycline showed changes in the MIC for either polymyxin B or tigecycline.

TABLE 1. Increased MIC of polymyxin B for CRKP isolates from three patients treated with polymyxin B

Isolate source	Sample type	Date of isolation (mo/day/yr)	Duration of treatment (days)	Polymyxin B MIC (μg/ml)
Patient 1	Peritoneal fluid	3/28/2006	14	1.5
	Blood	4/13/2006		32
Patient 2[a]	CSF[b]	11/5/2005	21[c]	0.75
	Blood	11/26/2005		12
Patient 3	Blood	12/7/2005	5	0.75
	Blood	12/12/2005		1,024

[a] Received polymyxin B intrathecally and intravenously in earlier course of therapy.
[b] CSF, cerebrospinal fluid.
[c] Polymyxin was started on the day of initial isolate recovery as an empirical therapy based on prior surveillance cultures yielding CRKP.

This observation may be explained by the emergence of resistance in the same strain or reinfection with a resistant strain from a heterogeneous bacterial population under antibiotic selective pressure. The clonal relationship between the initial and subsequent isolates was not examined in this study. The mechanisms of resistance to polymyxin in *K. pneumoniae* may be mediated by modification of the lipopolysaccharide of the outer membrane or by increased production of capsular polysaccharide (5, 13). These molecular changes induced by mutation are known to confer a low level of resistance and occur slowly (3, 5), in contrast to our cases in which isolates showed a significantly increased MIC in a relatively short period of time: 14 days, 21 days, and 5 days, respectively (Table 1). Reinfection with a resistant strain under antibiotic selective pressure might be implicated in these cases, and perhaps combination therapy with polymyxin B and tigecycline might have prevented this in patients who received both agents.

Although polymyxin and tigecycline do not show a synergistic effect on *K. pneumoniae* in vitro (10), they are not antagonistic and may have an additive effect when used together (9). Of importance is that combination therapy may prevent the emergence of resistance in CRKP isolates. Further studies are needed to determine the clinical significance of our findings and to evaluate the in vivo efficacy of combination therapy with polymyxin and tigecycline for treatment of CRKP infection.

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