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In Vitro Antimicrobial Activity and Mutant Prevention Concentration of Colistin against *Acinetobacter baumannii*[▽]

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The antimicrobial activities of colistin and other antibiotics against clinical *Acinetobacter baumannii* and the mutant prevention concentration (MPC) of colistin against multidrug-resistant *A. baumannii* were studied. All 70 stains tested were sensitive to colistin. The MPC range of colistin against 30 multidrug-resistant *A. baumannii* stains was approximately 32 to >128 μg/ml, and the MPC at which 90% of the isolates tested were prevented (MPC_{90}) exceeded 128 μg/ml, which was much higher than the plasma concentration of colistin at the current recommended dosage. So, combination therapy for colistin treatment of *A. baumannii* would be prudent to slow the emergence of resistance.

Acinetobacter baumannii has emerged as a highly troublesome pathogen for many institutions globally. The epidemic potential and the clinical severity of *A. baumannii* infections are primarily related to multidrug-resistant (MDR) and pan-resistant strains (6). Colistin exhibits rapid and concentration-dependent bactericidal activity, but it was largely replaced by aminoglycosides in the 1970s because of its nephrotoxicity and neurotoxicity. In the past 10 to 15 years, however, colistin has reappeared for "salvage" therapy with infections caused by MDR Gram-negative bacteria (4). Unfortunately, colistin-resistant strains have emerged (5), and the treatment of MDR *Pseudomonas aeruginosa* and *A. baumannii* infections has become more difficult. In this study, we evaluated the antibacterial activities of four kinds of antibiotics commonly used against clinically isolated *A. baumannii.* In addition, mutant prevention concentrations (MPCs) of colistin for strains identified as MDR *A. baumannii* were evaluated. The results help explain the occurrence of colistin-resistant strains.

A total of 70 *A. baumannii* clinical isolates were selected during the period from January 2006 to June 2007 from 3 general hospitals in the urban area of Beijing, China. Piperacillin, ceftazidime, cefepime, meropenem, netilmicin, amikacin, ciprofloxacin, and levofloxacin standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China (NICPBP; Beijing, China). Ampicillin-sulbactam, piperacillin-sulbactam, cefoperazone-sulbactam, and imipenem-cilastatin were purchased from Shandong Lukang Pharmaceutical Co., Ltd. (Shandong, China), Hayao Group (Heilongjiang, China), Guangzhou Baiyunshan Tianxin Pharmaceutical Co., Ltd. (Guangdong, China), and Shenzhen Haibin Pharmaceutical Co., Ltd., respectively. Colistin was purchased from Sigma-Aldrich (036K1374; St. Louis, MO).

MICs were measured by broth dilution assay (Mueller-Hinton [M-H] broth; Difco). The initial concentration for each bacterial suspension was 1.5×10^5 CFU/ml. The final concentrations for all the antibiotics mentioned above ranged from 0.125 to 128 μg/ml. Serial 2-fold dilutions of each drug were prepared. Results were obtained after plates were incubated at 37°C for 24 h. MIC_{90}s and MIC_{50}s were determined as the lowest drug concentrations that inhibited the growth of the tested strains by 90% and 50%, respectively.

MPCs were determined as described by Zhao et al., with some modifications (7). Each strain was inoculated onto an M-H agar plate and incubated at 37°C overnight. Bacterial cells were collected from these plates, transferred to 400 ml M-H broth (Difco), and incubated at 37°C overnight, followed by a 10-fold dilution and 6 h of incubation with shaking at 37°C. Then, the bacterial suspension was cooled on ice, and the bacterial cells were collected by centrifugation at 4°C immediately. The cells were washed twice with broth medium and resuspended in a small amount of broth, resulting in bacterial concentrations of about 3×10^{10} CFU/ml. Next, 100-μl aliquots of suspension were plated onto an M-H agar (Difco) plate containing various concentrations of antibiotics. MPCs were determined to be the lowest antibacterial concentrations that completely inhibited bacterial growth after incubation at 37°C for 72 h.

Table 1 lists the antibiotic susceptibility levels of 70 *A. baumannii* clinical isolates. The resistance rates of these strains to penicillins and cephalosporins, carbapenems (meropenem and imipenem-cilastatin), aminoglycosides (netilmicin and amikacin), and fluoroquinolones (ciprofloxacin and levofloxacin) were 71.4% to 82.9%, 75.7% to 77.1%, 71.4% to 75.7%, and 32.8% to 82.9%, respectively. The susceptibility rate for colistin was 100%, and no colistin-resistant strain was found.

Isolates resistant to 3 or more different types of antibiotics tested were defined as MDR strains. Thirty MDR *A. baumannii* strains were selected for an MPC assay of colistin (7). The range of MPCs was from 32 to >128 μg/ml, and the MPC at which 90% of the isolates tested were prevented (MPC_{90}) was greater than 128 μg/ml. Except for 1 strain with a MIC of 32 μg/ml and another 5 strains with MPCs of 64 μg/ml, the MPCs of the other 24 strains were equal to or greater than 128 μg/ml. Moreover, 24 of the 30 strains tested had MPC/MIC values equal to or greater than 128 μg/ml.

All the isolates in this study were sensitive to colistin, in-

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TABLE 1. Antibiotic susceptibilities of 70 *A. baumannii* strains studied

Antibiotic	MIC (μg/ml)			Susceptible (%)[a]	Intermediate (%)[a]	Resistant (%)[a]
	Range	50%	90%			
Ampicillin-sulbactam	2–>128	128	>128	17.2	1.4	81.4
Piperacillin	8–>128	>128	>128	10.0	7.1	82.9
Piperacillin-sulbactam	2–>128	>128	>128	17.2	11.4	71.4
Cefoperazone-sulbactam	2–>128	128	>128	17.2	1.4	81.4
Ceftazidime	2–>128	>128	>128	17.2	5.7	77.1
Cefepime	1–>128	>128	>128	15.7	2.9	81.4
Meropenem	0.5–128	32	64	22.9	0	77.1
Imipenem-cilastatin	0.25–>128	16	128	21.4	2.9	75.7
Netilmicin	0.5–>128	>128	>128	20.0	4.3	75.7
Amikacin	0.5–>128	>128	>128	28.6	0	71.4
Ciprofloxacin	0.064–>128	16	64	15.7	1.4	82.9
Levofloxacin	0.0313–32	4	16	38.6	28.6	32.8
Colistin	0.5–2	1	1	100	NA	0

[a] MIC interpretive standards were determined according to those of the Clinical and Laboratory Standards Institute (CLSI, 2009). NA, not applicable.

cluding MDR strains. Thus, colistin can serve as the last resort for infections caused by MDR *A. baumannii* strains. The desirable bactericidal activity of colistin is due mainly to the properties of colistin itself. However, the fact that colistin has been withdrawn from clinical practice in China for a long time due to adverse renal and neurological effects might be another important contributing factor. The emergence of colistin-resistant *A. baumannii* has already been experienced after years of reuse in other countries (5). Our MPC assay results may help explain the generation of resistant strains. As we know, the MPC is the upper boundary of the mutant selection window (MSW), with MSW referring to an antimicrobial concentration range extending from the minimal concentration required to block the growth of wild-type bacteria to that required to inhibit the growth of the least susceptible, single-step mutant. Maintaining antimicrobial concentrations inside the window is expected to selectively enrich resistant mutant subpopulations, whereas keeping concentrations above the window is expected to restrict selective enrichment (1). Our study showed that the MPCs of colistin to 30 MDR *A. baumannii* isolates were equal to or greater than 128 μg/ml, and the range of MSWs was quite wide (approximately 1 to 128 μg/ml).

The recommended dose regimen of colistin methanesulfonate was approximately 4 to 6 mg/kg/day intravenously (i.v.) or intramuscularly (i.m.) for patients who weigh 60 kg or less with normal renal function and approximately 240 to 480 mg/day (United Kingdom) or 720 mg/day (United States) in three divided doses i.v. or i.m. for patients who weigh more than 60 kg with normal renal function (2). Clinical data showed that after i.v. administration of 225 mg colistin methanesulfonate every 8 or 12 h for at least 2 days, the maximum concentration of drug in serum (C_{max}) and minimum concentration of drug in serum (C_{min}) were 2.93 and 1.03 μg/ml, respectively, at steady state (3). This indicated that at the recommended dosage, the concentration of colistin just fell into the MSW, which is expected to enrich resistant mutant subpopulations. To prevent mutant selection, combination therapy might be the only choice, because an increase of the colistin concentration in plasma would lead to a high risk of nephrotoxicity and neurotoxicity. Two or more antimicrobial agents might drastically narrow the MSW range if their normalized pharmacokinetic profiles superimpose at concentrations that inhibit bacterial growth.

In conclusion, our consideration of the MPC, the mutant selection window, and drug pharmacokinetics leads us to believe that combination therapy for colistin treatment of *A. baumannii* would be prudent to slow the emergence of resistance. Moreover, our data add the colistin-*A. baumannii* combination to the list of those for which the MPC can be readily measured.

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