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Mutant prevention concentration of colistin alone and in combination with levofloxacin or tobramycin against multidrug-resistant *Acinetobacter baumannii*

Sir,

Infection due to multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) is an increasingly significant problem in modern health care. This pathogen has been implicated in a wide range of infections at most anatomical sites, ranging in severity from asymptomatic to fulminant sepsis. Almost all available antibiotics, including β-lactams, fluoroquinolones, tetracyclines and aminoglycosides, have lost their activity against MDR-Ab. An older drug, colistin, has been re-used in the clinic as the last-resort treatment. Unfortunately, the number of clinical reports of colistin resistance has risen rapidly in parallel with its increasing use [1]. Bacterial exposure during currently recommended colistin dosage regimens is inadequate to prevent the emergence of resistance. The mutant prevention concentration (MPC) is the drug concentration required to prevent the emergence of mutant colonies, proposed by Drlica in 2003 [2]. We previously evaluated MPCs of colistin against 30 MDR-Ab and found that concentrations exceeding 128 μg/mL prevented mutation in >90% of all isolates [3]. The mutant selection window (MSW) is very wide, from 1 μg/mL to 128 μg/mL. These results indicated that colistin-resistant strains are easily selected at the recommended dosage of colistin.

In this study, a total of 73 clinically identified MDR-Ab epidemic strains were selected during the period April–July 2010 from two hospitals in Beijing, China. A threshold level of 87% was chosen for the establishment of clonal relatedness, and the isolates could be grouped into six pulsed-field gel electrophoresis (PFGE) clonal types (data not shown). Minimum inhibitory concentrations (MICs) of colistin to these strains were 0.5 μg/mL, except for AB42 that showed intermediate sensitivity to cefepime but was resistant to all other antibiotics (including colistin, with a MIC of 8 μg/mL). Six strains belonging to six separate PFGE clusters were selected for the following combination MPC study (Table 1).

Each strain was inoculated onto Mueller–Hinton agar (MHA) plates and grown overnight at 37 °C in ambient air. Overnight growth was swabbed into 20 mL of Mueller–Hinton broth (MHB) and was incubated at 37 °C overnight. Bacterial suspensions were transferred into 200 mL of MHB followed by 6 h of incubation with shaking at 37 °C in ambient air in order to achieve an inoculum of ca. 3×10^{10} CFU/mL. Samples of 100 μL were plated onto MHA plates containing 1×, 2×, 4×, 8× and 16× MIC of colistin alone or in combination with either levofloxacin or tobramycin at different concentrations (1, 2, 4, 8, 16 and 32 μg/mL). Drug-containing plates were incubated at 37 °C for 72 h to allow time for slow-growing colonies to be recovered. Resistant mutants were confirmed by regrowth on agar containing antibiotic at the concentration used to select the colonies. Alone or combination MPCs were determined as the lowest drug or drug combination concentrations that prevented recovery of colonies. The MPC of levofloxacin and tobramycin was not tested alone as most strains show high resistance to these agents.

Table 1
MICs and MPC/MIC ratios for colistin (COL) alone or in combination with levofloxacin (LVX) or tobramycin (TOB) in six different PFGE clone types of multidrug-resistant *Acinetobacter baumannii*.[a]

Isolate No.	MIC (μg/mL)			MPC/MIC		
	COL	LVX	TOB	COL	COL + LVX	COL + TOB
AB3	0.5	2	128	64	8[b]	8[c]
AB19	0.5	32	128	>64	16[b]	8[c]
AB34	0.5	8	>128	>64	16[d]	16[c]
AB42	8	16	>128	>8	2[e]	2[f]
AB57	0.5	8	4	64	8[e]	8[c]
AB67	0.5	16	2	>64	16[e]	16[c]

MIC, minimum inhibitory concentration; MPC, mutant prevention concentration; PFGE, pulsed-field gel electrophoresis.
[a] The MPC/MIC ratio is defined as the ratio of the MPC obtained to the original MIC.
[b] Levofloxacin 2 μg/mL.
[c] Tobramycin 2 μg/mL.
[d] Levofloxacin 4 μg/mL.
[e] Levofloxacin 8 μg/mL.
[f] Tobramycin 8 μg/mL.

The MPC/MIC ratio was used to represent the data obtained. All MPC experiments were carried out in triplicate on separate days. The MPC/MIC ratio of colistin tested alone was 64 or >64 for colistin-sensitive MDR-Ab, supporting our previous finding of a wide MSW of colistin alone against *A. baumannii* [3]. Following the addition of levofloxacin or tobramycin at relatively low concentrations (2 μg/mL or 4 μg/mL), the MPC/MIC decreased 4- to 8-fold and colistin-resistant *A. baumannii* mutants were not selected. For colistin-resistant strain AB42, the MPC/MIC of colistin alone was >8. A 4-fold decrease was seen after combination with levofloxacin or tobramycin at 8 μg/mL (Table 1).

Zhanel et al. showed that compared with colistin alone, combination of levofloxacin with colistin resulted in lower mutation frequencies in *Pseudomonas aeruginosa* [4]. This combination in the current study also proved to be efficient at preventing selection of colistin resistance in MDR-Ab. At the recommended dosage of 1000 mg, levofloxacin can reach a maximum plasma concentration of 15.55 μg/mL, with good tolerance [5]. Thus, the combination of colistin and levofloxacin has great potential to become a treatment option against MDR-Ab in the clinic. It should be mentioned that the combination strategies are also efficient against colistin-resistant strains (e.g. AB42). This is also the first report demonstrating that clinically isolated colistin-resistant *A. baumannii* is sensitive to the combination of tobramycin and colistin. However, we are not clear whether the regimen will be associated with a higher safety risk in vivo as both agents have potential nephrotoxicity.

In conclusion, these studies demonstrated that combination of colistin with levofloxacin or tobramycin in vitro can narrow its MSW against MDR-Ab and prevent the emergence of colistin-resistant strains. As the emergence of colistin-resistant *A. baumannii* strains is increasingly a clinical problem, the strategy of combination therapy with agents with different mechanisms of action may slow the development of resistance. Further research in vivo is required to determine whether these effects would also be expected in patients.

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