ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2009, p. 3599–3601 Vol. 53, No. 8
0066-4804/09/$08.00+0 doi:10.1128/AAC.00641-09

Letters to the Editor

In Vitro Activity of NXL104 in Combination with β-Lactams against *Klebsiella pneumoniae* Isolates Producing KPC Carbapenemases▽

Klebsiella pneumoniae isolates producing class A KPC carbapenemases (KPC-Kp) are spreading at an alarming rate around the world (8, 10, 11). These isolates are highly resistant to penicillins, cephalosporins, and commercially available β-lactam/β-lactamase inhibitor combinations and show reduced susceptibility to carbapenems. KPC-Kp are also commonly resistant to quinolones, aminoglycosides, and occasionally to colistin (3, 7, 10). Therefore, our antibiotic choices for the treatment of infections due to KPC-Kp isolates are extremely limited.

Developing novel β-lactamase inhibitors that are active against different classes of carbapenemases is an important goal (1). NXL104 (Novexel SA, Romainville, France) is a new β-lactamase inhibitor currently in clinical trials (http://clinicaltrials.gov/) and active against class A (e.g., TEM-, SHV-, and CTX-M-types) and class C β-lactamases (2, 9). However, data regarding its in vitro activity in combination with β-lactams against KPC-Kp isolates are very limited (9).

In the present work, we analyzed the in vitro activity of NXL104 in combination with different β-lactams against a collection of 42 well-characterized KPC-Kp clinical isolates collected in the United States (6, 7). In a previous analysis, we demonstrated that (i) these strains possessed a complex β-lactamase background (i.e., three or more *bla* genes per isolate) and that (ii) clavulanate or tazobactam were unable to lower the MICs of β-lactams to susceptibility ranges for these strains (7).

MICs for β-lactams and β-lactams plus NXL104 at three different constant concentrations (i.e., 1, 2, and 4 μg/ml) were determined by using the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) criteria, on cation-adjusted Mueller-Hinton agar (BBL, Becton Dickinson, Sparks, MD) using a Steers replicator (4). We tested piperacillin, cefotaxime, ceftazidime (Sigma Chemical Co.), cefepime, and aztreonam (Bristol-Myers Squibb, Princeton, NJ). NXL104 was a kind gift of Dr. Christine Miossec (Novexel). ATCC strains *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, and *K. pneumoniae* 700603 were used as controls. Susceptibility results, including those for the combinations with NXL104, were interpreted according to the CLSI criteria established for the β-lactams when tested alone (5).

As shown in Table 1, KPC-Kp isolates were very resistant to all noncarbapenem β-lactams tested (overall, MIC_{90} values were ≥128 μg/ml). In contrast, MICs for the combination of NXL104 at a constant concentration of 4 μg/ml with piperacillin, extended-spectrum cephalosporins, or aztreonam were in the susceptible range for all strains (overall, MIC_{90} values were ≤2 μg/ml). All KPC-Kp strains were also susceptible to β-lactams plus NXL104 at a constant concentration of 2 μg/ml (overall, MIC_{90} values were ≤8 μg/ml). Additionally, NXL104 used at a concentration of 1 μg/ml was very effective at lowering MICs when combined with a cephalosporin or aztreonam (Table 1).

In conclusion, we demonstrate that NXL104 can effectively lower the MIC of β-lactams when tested against contemporary KPC-Kp clinical isolates. The combination of NXL104 with extended-spectrum cephalosporins or aztreonam could represent a promising therapeutic strategy to treat infections due to KPC-Kp isolates. Further studies to evaluate the activity of NXL104 in combination with investigational β-lactams should be performed against large collections of gram-negative bacilli producing different classes of carbapenemases.

R.A.B. is supported by the National Institutes of Health (grant RO1-AI063517), the Veterans Affairs Merit Review Program, and the Geriatric Research Education and Clinical Center, VISN 10. We thank Drs. Louis B. Rice, David L. Paterson, Michael R. Jacobs, Gerri S. Hall, and Stephen G. Jenkins for providing *K. pneumoniae* isolates.

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TABLE 1. Antimicrobial susceptibility test results for the 42 *K. pneumoniae* isolates producing the KPC carbapenemase collected in United States

Antimicrobial or combination	MIC (μg/ml) distribution of KPC-Kp isolates [no. (%)]														MIC_{50}	MIC_{90}	% S[a]
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512			
Piperacillin														42 (100)	≥512	≥512	0.0
+ NXL104 (4 μg/ml)	13 (31.0)	1 (2.4)	2 (4.8)	5 (11.9)	13 (31.0)	8 (19.0)									0.5	2	100
+ NXL104 (2 μg/ml)					1 (2.4)	2 (4.8)	10 (23.8)	27 (64.3)	2 (4.8)						8	8	100
+ NXL104 (1 μg/ml)							2 (4.8)	8 (19.0)	24 (57.1)	8 (19.0)					16	32	81.0
Cefotaxime									1 (2.4)	6 (14.3)	17 (40.4)	7 (16.7)	6 (14.3)	5 (11.9)	64	≥512	0.0
+ NXL104 (4 μg/ml)	11 (26.2)	14 (33.3)	13 (31.0)	4 (9.5)											0.125	0.25	100
+ NXL104 (2 μg/ml)	7 (16.7)	11 (26.2)	10 (23.8)	14 (33.3)											0.25	0.5	100
+ NXL104 (1 μg/ml)	6 (14.3)	10 (23.8)	9 (21.4)	9 (21.4)	6 (14.3)	1 (2.4)	1 (2.4)								0.25	1	100
Ceftazidime										1 (2.4)	3 (7.1)	7 (16.7)	8 (19.0)	23 (54.8)	≥512	≥512	0.0
+ NXL104 (4 μg/ml)	11 (26.2)	5 (11.9)	15 (35.7)	4 (9.5)	7 (16.7)										0.25	100	100
+ NXL104 (2 μg/ml)				3 (7.1)	3 (7.1)	17 (40.5)	14 (33.3)	5 (11.9)							2	8	100
+ NXL104 (1 μg/ml)					1 (2.4)	3 (7.1)	15 (35.7)	19 (45.2)	4 (9.5)						8	8	90.5
Cefepime								3 (7.1)	15 (35.7)	12 (28.6)	2 (4.8)	8 (19.0)	2 (4.8)		32	128	7.1
+ NXL104 (4 μg/ml)	34 (81.0)	4 (9.5)	4 (9.5)												≤0.06	0.125	100
+ NXL104 (2 μg/ml)	8 (19.0)	11 (26.2)	7 (16.7)	10 (23.8)	5 (11.9)	1 (2.4)									0.25	1	100
+ NXL104 (1 μg/ml)	5 (11.9)	10 (23.8)	8 (19.0)	9 (21.4)	9 (21.4)		1 (2.4)								0.25	1	100
Aztreonam												1 (2.4)	4 (9.6)	37 (88.1)	≥512	≥512	0.0
+ NXL104 (4 μg/ml)	40 (95.2)		2 (4.8)												≤0.06	≤0.06	100
+ NXL104 (2 μg/ml)	7 (16.7)	28 (66.7)	1 (2.4)	6 (14.3)											0.125	0.5	100
+ NXL104 (1 μg/ml)			15 (35.7)	14 (33.3)	9 (21.4)	3 (7.1)	1 (2.4)								0.5	1	100

[a] S, susceptible. Interpretation according to CLSI criteria established for β-lactam alone (5): piperacillin (S ≤ 16 μg/ml); cefotaxime, ceftazidime, cefepime, and aztreonam (S ≤ 8 μg/ml).

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▽ Published ahead of print on 15 June 2009.