In Vitro Activity of Ceftazidime-NXL104 against 396 Strains of β -Lactamase-Producing Anaerobes[∇]

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NXL104, a novel β -lactamase inhibitor, was tested at a constant concentration of 4 μ g/ml in combination with ceftazidime (CAZ), with and without added metronidazole, against 396 β -lactamase-producing strains of anaerobic bacteria. MIC₅₀/MIC₉₀ values for *Bacteroides fragilis* and the *B. fragilis* group were 8/16 and 64/>128 μ g/ml, respectively. Although CAZ-NXL104 had limited activity against most anaerobic strains, in combination with metronidazole it shows potential for treating mixed infections involving resistant *Enterobacteriaceae* and anaerobes.

The worldwide emergence of antimicrobial resistance in *Enterobacteriaceae* containing extended-spectrum β -lactamases (ESBLs), metallo-carbapenemases, and KPC enzymes has radically altered clinicians' empirical selection of antimicrobial agents (3, 6, 10), and new alternative therapeutic agents are urgently needed to treat these serious infections. These multidrug-resistant organisms can also be part of mixed infections, such as intraabdominal and diabetic foot infections, where anaerobic bacteria are important pathogens with their own unique resistance issues. NXL104 is a novel β -lactamase inhibitor with demonstrated activity against AmpC, TEM, SHV, and CTX-M β -lactamases as well as non-metallo-carbapenemases (6, 10); combined with ceftazidime, it is currently in development for treatment of serious intraabdominal infections. While NXL104 has been shown to be effective *in vitro* in combination with various cephalosporins against these resistant aerobic organisms, there is scant data on its activity against the anaerobic bacteria (6, 10). Consequently, we tested ceftazidime (CAZ) and NXL104 alone and in combination against 396 clinical strains of anaerobic bacteria that are often encountered in mixed infections, selected on the basis of various MIC levels (determined previously) for β -lactamase inhibitor combinations, with an emphasis on the less susceptible strains. CAZ-NXL104 was further tested in combination with various concentrations of metronidazole to look for potential synergy.

The test strains included *Bacteroides* spp., *Parabacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., *Bilophila wadsworthia*, *Desulfovibrio* spp., *Campylobacter* spp., *Fusobacterium* spp., *Clostridium clostridioforme* group spp., and *Eggerthella lenta*, recovered from patients with primarily intraabdominal infections, identified by standard methods (5, 7). Test strains were stored at -70°C in 20% skim milk. The individual species are listed in footnotes to Tables 1 and 2. For strains that could not be identified by conventional techniques, partial sequenc-

ing of 16S rRNA genes was performed as described elsewhere (12).

Comparator drugs included amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, metronidazole, clindamycin, and imipenem. The antimicrobial agents were reconstituted according to their manufacturers' instructions and stored at -70°C (1). NXL104 was added at a constant concentration of 4 μ g/ml to serial dilutions of ceftazidime. MICs are reported for ceftazidime.

The agar dilution test was conducted according to the procedures in CLSI approved standard M11-A7 (1). Serial dilutions of each compound were prepared and added to molten agar to prepare the plates. Three extra dilution series of CAZ-NXL104 with the addition of 1, 0.5, 0.25, or 0.125 μ g/ml of metronidazole to each tube of molten agar were prepared to test for possible interactions. Prior to testing, the strains were taken from the freezer and transferred at least twice on supplemented brucella blood agar to ensure purity and good growth. On the day of testing, inocula were prepared in the anaerobic chamber from 48-h plates by suspending organisms into brucella broth to the turbidity of the 0.5 McFarland standard. The inocula were pipetted into the wells of a Steers replicator and applied to the test plates for a final inoculum of $\sim 10^5$ CFU/spot. Control strains *Bacteroides fragilis* ATCC 25285, *Klebsiella pneumoniae* ATCC 700603, and *Escherichia coli* ATCC 25922 were included each day of testing. (The aerobic organisms were used because they have CLSI-established quality control ranges for ceftazidime and NXL104.) All plates were incubated in the anaerobic chamber at 37°C for 44 h and then examined for growth. The MIC was the drug concentration that completely inhibited growth or resulted in a major reduction of growth compared to the drug-free growth control (1).

Table 1 shows the MIC distribution and cumulative percent at each value for the *Bacteroides*, *Parabacteroides*, and *Prevotella* species against the β -lactamase inhibitor combinations, imipenem, clindamycin, CAZ-NXL104, metronidazole, and CAZ-NXL104 in combination with metronidazole at various concentrations relative to each strain's metronidazole MIC. The number of strains with metronidazole MICs that fell

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TABLE 1. MIC distributions of Gram-negative anaerobes selected for decreased susceptibility to β -lactams for ceftazidime-NXL104, other β -lactamase inhibitor combinations, metronidazole, imipenem, and clindamycin

Organism (no. of strains) and β -lactamase inhibitor ^a	Cumulative % of strains with MICs (μ g/ml) of:											
	≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
<i>Bacteroides caccae</i> (15)												
Ceftazidime							13	20	27			100
NXL104												100
Ceftazidime-NXL104						7	27	40	60	73	80	100
Metronidazole		7	27	87	100							
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC (n = 11)	29	36		43	57	64	79	93	100			
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC (n = 12)	85			100								
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC (n = 4)	100											
Amoxicillin-clavulanate 2:1			7	40	47	53	87	93		100		
Ampicillin-sulbactam 2:1				13	20	53	67	87	93	100		
Piperacillin-tazobactam	7	13	33	53	80	93						100
Imipenem	7	33	53	73	93					100		
Clindamycin	14	21		28	42		49					100
<i>B. fragilis</i> (68)												
Ceftazidime							7	22	40	56	66	100
NXL104												100
Ceftazidime-NXL104					15	44	57	90	94	97	99	100
Metronidazole	1	7	50	99	100							
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC (n = 67)		2	5	30	43	56	92	97	98			100
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC (n = 68)	80	86	91	94	95	97	100					
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC (n = 34)	100											
Amoxicillin-clavulanate 2:1			9	37	69	82	94	99		100		
Ampicillin-sulbactam 2:1			2	10	28	63	88	94	99	100		
Piperacillin-tazobactam	9	21	47	79	87		94			99		100
Imipenem	10	21	44	78	90	96	99			100		
Clindamycin	13	19	37	63	72	73						100
<i>B. ovatus</i> (38)												
Ceftazidime							13	20	27			100
NXL104												100
Ceftazidime-NXL104									16	37	68	100
Metronidazole		8	71	89	97	100						
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC (n = 37)						6	29	47	85	97	100	
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC (n = 34)	68	71		76		79	91	100				
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC (n = 26)	100											
Amoxicillin-clavulanate 2:1			16	34	40	53	71	97	100			
Ampicillin-sulbactam 2:1				8	34	42	76	90	100			
Piperacillin-tazobactam		5	16	32	61	76	48	97	100			
Imipenem	5	47	79	90	100							
Clindamycin	5	8	19	35	43				46			100
<i>Bacteroides stercoris</i> / <i>B. uniformis</i> / <i>Bacteroides salyersiae</i> (28) ^b												
Ceftazidime									11			100
NXL104											11	100
Ceftazidime-NXL104						4	7	18	29	64	93	100
Metronidazole	7	18	82	100								
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC (n = 25)						4	13	39	74	96	100	
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC (n = 26)	65			69		73	81	88	96	100		
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC (n = 21)	100											
Amoxicillin-clavulanate 2:1			11	25	39	71	96	100				

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TABLE 1—Continued

Organism (no. of strains) and β -lactamase inhibitor ^a	Cumulative % of strains with MICs (μ g/ml) of:											
	≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Ampicillin-sulbactam 2:1				18	21	36	86	93	100			
Piperacillin-tazobactam	11	14	29	68	86	96				100		
Imipenem		32	75	93		100						
Clindamycin	18	22	26	40	54	58					62	100
<i>B. thetaiotaomicron</i> (52)												
Ceftazidime												100
NXL104												100
Ceftazidime-NXL104								2		33	81	100
Metronidazole		6	48	87	100							
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC ($n = 50$)	2		4	6	8	10	27	47	76	92	100	
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC ($n = 45$)	95					98		100				
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC ($n = 25$)	100											
Amoxicillin-clavulanate 2:1			8	27	33	60	89	94	100			
Ampicillin-sulbactam 2:1				8	23	31	56	96	100			
Piperacillin-tazobactam		2		4		23	48	73	89	98	100	
Imipenem	14	39	77	90	98		100					
Clindamycin	2	4	14	22	24	44	54	56				100
<i>Bacteroides vulgatus</i> (23)												
Ceftazidime									4		8	100
NXL104												100
Ceftazidime-NXL104								26	61	83	100	
Metronidazole		22	61	91	100							
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC ($n = 18$)	28				39	44	61	83	94	100		
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC ($n = 21$)	63	74	79				100					
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC ($n = 14$)	100											
Amoxicillin-clavulanate 2:1					9	26	83	100				
Ampicillin-sulbactam 2:1						4	57	91	100			
Piperacillin-tazobactam				13	39	74	91	100				
Imipenem		4	39	83	96			100				
Clindamycin	30	47		51	60							100
<i>Parabacteroides</i> spp. (50) ^c												
Ceftazidime							2	4		14	22	100
NXL104												100
Ceftazidime-NXL104						4	22	64	80	90	94	100
Metronidazole	2	18	68	96	98	100						
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC ($n = 43$)	10	13	15		20	45	73	83	98			100
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC ($n = 48$)	87			89		96	98	100				
Ceftazidime-NXL104 plus 2 $\times$ MTZ MIC ($n = 34$)	100											
Amoxicillin-clavulanate 2:1				6	16	40	68	96	100			
Ampicillin-sulbactam 2:1				2	6	10	42	86	94	98	100	
Piperacillin-tazobactam				4	26	70	86	96				100
Imipenem	2	12	46	70	90	100						
Clindamycin	8		12	30	44	70	74	76			78	100
<i>Prevotella/Porphyromonas</i> spp. (49) ^d												
Ceftazidime			4	10	16	37	43	47	65	78	88	100
NXL104									12	26	43	100
Ceftazidime-NXL104	16	29	31	49	76	92	100					
Metronidazole	2	16	61	92	100							
Ceftazidime-NXL104 plus 0.5 $\times$ MTZ MIC ($n = 44$)	38	56	64	74	97	100						
Ceftazidime-NXL104 plus 1 $\times$ MTZ MIC ($n = 32$)	88	97	100									

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TABLE 1—Continued

Organism (no. of strains) and β -lactamase inhibitor ^a	Cumulative % of strains with MICs (μ g/ml) of:											
	≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Amoxicillin-clavulanate 2:1	16	39	49	65	90	100						
Ampicillin-sulbactam 2:1	4	27	45	65	82	100						
Piperacillin-tazobactam	100											
Imipenem	93	100										
Clindamycin	71		73	75							77	100

^a Ceftazidime-NXL104, NXL constant at 4 μ g/ml; piperacillin-tazobactam, tazobactam constant at 4 μ g/ml; *n*, the number of strains which had metronidazole MICs in range for the calculation.

^b *B. stercoris* (4 strains), *B. uniformis* (23 strains), *B. salyersiae* (1 strain).

^c *P. distasonis* (35 strains), *P. goldsteinii* (3 strains), *P. merdae* (12 strains).

^d *Prevotella bivia* (11 strains), *Prevotella buccae* (5 strains), *Prevotella corporis* (1 strain), *Prevotella denticola* (2 strains), *Prevotella disiens* (7 strains), *Prevotella intermedia* (7 strains), *Prevotella melaninogenica* (7 strains), *Prevotella loeschii* (1 strain), *Prevotella oris* (2 strains), *Porphyromonas levii* (1 strain), *Porphyromonas somerae* (5 strains).

within the calculated range is indicated. Table 2 shows the activity of CAZ and NXL104 alone and in combination against the other organisms tested.

CAZ and NXL104 alone were mostly inactive (overall MIC₉₀ for both was >128 μ g/ml). *B. fragilis* was the most

susceptible of the *Bacteroides* group to ceftazidime alone and in combination with NXL104, with CAZ-NXL104 MIC₅₀ and MIC₉₀ values of 8 and 16 μ g/ml, respectively. Most of the *Parabacteroides distasonis* group showed elevated MICs to all the β -lactamase inhibitor combinations and to imipenem, with

TABLE 2. MIC distributions of selected anaerobes with decreased susceptibility to β -lactams for ceftazidime, NXL104, and ceftazidime-NXL104

Organism (no. of strains) and β -lactamase inhibitor	Cumulative % of strains with MICs (μ g/ml) of:											
	≤ 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
<i>Bilophila wadsworthia</i> (20)												
Ceftazidime								5	20	85	95	100
NXL104	25					45	70	75				100
Ceftazidime-NXL104	45	50		65			70			80	100	
<i>Campylobacter</i> spp. (5) ^a												
Ceftazidime			20		40							100
NXL104							20			60		100
Ceftazidime-NXL104	20	40									80	100
<i>Clostridium</i> spp. (15) ^b												
Ceftazidime							7	14	20	60	67	100
NXL104												100
Ceftazidime-NXL104							7	14	27	60	87	100
<i>Desulfovibrio</i> spp. (9) ^c												
Ceftazidime											22	100
NXL104										11	33	100
Ceftazidime-NXL104										33	89	100
<i>Eggerthella lenta</i> (10)												
Ceftazidime												100
NXL104												100
Ceftazidime-NXL104												100
<i>Fusobacterium</i> spp. (10) ^d												
Ceftazidime			20	40	70	80			90			100
NXL104								10	20			100
Ceftazidime-NXL104		20	40	60	80				90			100
<i>Veillonella</i> species												
Ceftazidime							25				100	
NXL104												100
Ceftazidime-NXL104							25				100	

^a *C. gracilis* (4 strains), *C. rectus* (1 strain).

^b *C. boieae* (8 strains), *C. butyricum* (2 strains), *C. clostridioforme* (2 strains), *C. hathewayi* (2 strains), *Clostridium* species (1 strain).

^c *D. fairfieldensis* (8 strains), *D. desulfuricans* (1 strain).

^d *F. gonidiaformans* (1 strain), *F. mortiferum* (3 strains), *F. nucleatum* (3 strains), *F. varium* (3 strains).

the CAZ-NXL104 MIC₅₀ and MIC₉₀ values at 16 and 64 µg/ml, respectively. The strains that were susceptible to other β-lactamase inhibitor combinations were also more susceptible to CAZ-NXL104 (data not shown). The indole-positive strains, including *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, and *Bacteroides uniformis*, were the most resistant to ceftazidime and CAZ-NXL104, regardless of susceptibility to the other β-lactamase inhibitor combinations, with most MICs at ≥128 µg/ml. For all members of the *B. fragilis* group, the MIC₅₀ and MIC₉₀ values were 64 and >128 µg/ml, respectively. For *Prevotella* and *Porphyromonas* spp., the MIC₅₀ and MIC₉₀ values were 2 and 4 µg/ml, respectively. For many of the *Bacteroides fragilis* and *Prevotella* strains, the addition of metronidazole at 0.5× or 1× their MIC resulted in a further reduction in the MIC of CAZ-NXL104 by one or more dilutions for some strains. But even at its original MIC, the metronidazole MICs for several organisms (*B. fragilis*, 3 strains; *B. ovatus*, 7 strains; *B. thetaiotaomicron*, 2 strains; *B. uniformis*, 9 strains; *P. distasonis*, 2 strains) appeared higher by 1 to 4 dilutions, suggesting possible antagonism. However, upon further examination, the MICs at the next-higher metronidazole concentration for those strains were ≤0.125 µg/ml, indicating that there were likely minor differences in MIC test interpretations and not antagonism between the drugs. This is shown in Table 1. For most strains, the effect was additive or indifferent.

NXL104 alone showed activity of ≤4 µg/ml against 9 of 20 strains of *Bilophila wadsworthia*, and CAZ-NXL104 was more active than either drug alone, with MICs of ≤1 µg/ml for 13 of the 20 strains. Two of the *Campylobacter* species were susceptible to ≤0.25 µg/ml CAZ-NXL104. Among the other organisms, 8 of 10 strains of *Fusobacterium* (the exception was 2 strains of *F. mortiferum*) were inhibited by ≤2 µg/ml of CAZ-NXL104. NXL104 did not increase the activity of ceftazidime against β-lactamase-producing strains of the *Clostridium clostridioforme*, *Clostridium bolteae*, *Clostridium butyricum*, *Desulfovibrio* spp., penicillin-resistant strains of *Veillonella*, or the cephalosporin-resistant strains of *Eggerthella lenta*.

Overall, piperacillin-tazobactam was the most active of all the β-lactamase inhibitor combinations tested, with only 1 strain each of *B. fragilis* and *B. thetaiotaomicron* and two strains of *P. distasonis* resistant, with MICs of ≥128 µg/ml, and 7 other strains with intermediate MICs of 64 µg/ml. Sixty-eight percent of all *Bacteroides* strains were fully susceptible to ampicillin-sulbactam at a breakpoint of 8 µg/ml compared to 59% for amoxicillin-clavulanate at a breakpoint of 4 µg/ml. Predictably, metronidazole showed the greatest activity against the anaerobes tested, with overall MIC₅₀ and MIC₉₀ values of 0.5 and 1 µg/ml, respectively. Clindamycin resistance was present in 52% of the *Bacteroides fragilis* group species, 30% of the *Parabacteroides* species, and 28% of *Bacteroides fragilis* strains. Imipenem resistance was present in only five isolates of *Bacteroides* species, but many showed elevated MICs of 2 to 4 µg/ml (Table 1). These included 12 *B. fragilis* strains, 16 other *B. fragilis* group species, and 13 *Parabacteroides* species.

Resistance to beta-lactam antibiotics can occur through a variety of mechanisms, both for aerobic Gram-negative rods, especially the *Enterobacteriaceae*, and for anaerobic bacteria.

For anaerobes, the most common resistance mechanism is β-lactamase production, as detected by the presence of genes such as *cepA* or *cfiA*, which also codes for carbapenemase (2, 4, 8, 9, 11). Other mechanisms include reduced penetration of the antibiotic into the periplasmic space by loss of porins or alterations in the penicillin binding proteins; efflux pumps may also play an important role in certain strains (13). Some organisms contain several resistance mechanisms and can be multiresistant to a wide range of beta-lactam antibiotics. Thus, the MIC range for β-lactam antibiotics against *Bacteroides* spp. is quite variable and cannot be explained by any one mechanism.

Strains selected for this study included those with reduced susceptibility to the standard β-lactamase inhibitor combinations to see if NXL104 would show enhanced activity in counteracting their β-lactamases. While there are no anaerobic breakpoints established for ceftazidime, the breakpoint for full susceptibility for other third-generation cephalosporins is ≤16 µg/ml (1). CAZ-NXL104 was remarkably active against *B. fragilis*, with 61 of 68 (90%) strains inhibited by ≤16 µg/ml. Although CAZ-NXL104 showed limited activity against most of the strains, in combination with metronidazole, CAZ-NXL104 is potentially effective for treating serious polymicrobial infections containing multidrug-resistant *Enterobacteriaceae* and anaerobic Gram-negative organisms.

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