In Vitro Activity of Ceftazidime Combined with NXL104 versus *Pseudomonas aeruginosa* Isolates Obtained from Patients in Canadian Hospitals (CANWARD 2009 Study)[▽]

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The *in vitro* activity of ceftazidime in combination with NXL104 versus 470 *Pseudomonas aeruginosa* clinical isolates was evaluated using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods. Ceftazidime had MIC₉₀s of 8 µg/ml and 32 µg/ml in the presence and absence of NXL104, respectively. Of 25 multidrug-resistant *P. aeruginosa* isolates, the percentages with a ceftazidime MIC of ≤8 µg/ml with and without NXL104 were 60% and 4%, respectively. These data suggest that the ceftazidime-NXL104 combination may prove useful for treating many *P. aeruginosa* infections.

Pseudomonas aeruginosa is an important cause of nosocomial respiratory, urinary tract, bloodstream, and wound infections (2, 7, 8, 11). In recent years, *P. aeruginosa* clinical isolates resistant to multiple classes of antimicrobial agents have become increasingly common (9, 14). The treatment of serious infections caused by these resistant isolates poses a therapeutic challenge for clinicians, who are often faced with extremely limited antimicrobial options from which to choose. Not surprisingly, multidrug resistance among *P. aeruginosa* has been associated with adverse clinical outcomes, including increased mortality (1, 10, 19). Of concern, there are few new antimicrobial agents currently under development that have significant activity versus *P. aeruginosa* (4).

NXL104 (formerly AVE1330A) is a novel non-β-lactam β-lactamase inhibitor currently in clinical development (17, 18). This agent demonstrates a broad spectrum of inhibitory activity *in vitro* versus Ambler class A and class C β-lactamase enzymes (3, 13, 18). Resistance to β-lactam antimicrobials among *P. aeruginosa* isolates is mediated, in part, by production of an AmpC enzyme (an Ambler class C β-lactamase) (9). In theory, combination of NXL104 with an antipseudomonal β-lactam antimicrobial such as ceftazidime may result in improved activity of the corresponding β-lactam versus *P. aeruginosa*. The purpose of this study was to evaluate the *in vitro* activity of ceftazidime in combination with NXL104 in comparison with that of other antipseudomonal antimicrobials against *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals.

Fifteen tertiary-care medical centers representing 8 of the 10 Canadian provinces submitted pathogens obtained from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units (CANWARD 2009

study). The sites were geographically distributed in a population-based fashion. From January through December 2009, inclusive, each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) obtained from inpatients and outpatients with bloodstream (*n* = 165), respiratory (*n* = 100), urine (*n* = 50), and wound (*n* = 50) infections. The medical centers submitted clinically significant isolates, as defined by their local site criteria. Isolate identification was performed by the submitting site and confirmed at the reference site as required (i.e., when morphological characteristics and antimicrobial susceptibility patterns did not fit the reported identification). Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at –80°C until MIC testing was carried out.

Following two subcultures from frozen stock, the *in vitro* activity of commonly used antipseudomonal antimicrobials was determined by broth microdilution, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (5, 6). NXL104 was obtained from Novexel, France (now owned by AstraZeneca, United Kingdom). A fixed concentration of 4 µg/ml NXL104 was evaluated in combination with doubling concentrations of ceftazidime. Ten randomly selected ceftazidime-susceptible *P. aeruginosa* isolates were also evaluated versus doubling concentrations of NXL104 in the absence of ceftazidime. Antimicrobial MIC interpretive standards were defined according to CLSI breakpoints (6). For doripenem, breakpoints defined by the U.S. Food and Drug Administration (FDA) were used (15). At present, no breakpoints have been set for the combination of NXL104 and ceftazidime. Multidrug-resistant (MDR) *P. aeruginosa* isolates were defined as isolates demonstrating resistance to at least one antimicrobial agent from three or more different classes. For the purpose of this report, the antimicrobial classes considered were aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), cefepime or piperacillin-tazobactam (considered together), and carbapenems (mero-

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TABLE 1. Antimicrobial susceptibility of 470 *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals in 2009^a

Antimicrobial	All isolates (<i>n</i> = 470)							% S for MDR isolates (<i>n</i> = 25)
	Breakpoint interpretation (%)			MIC (µg/ml)		MIC range (µg/ml)		
	S	I	R	MIC ₅₀	MIC ₉₀	Min	Max	
Amikacin	90.2	3.6	6.2	8	16	≤1	>64	44
Cefepime	81.3	12.6	6.2	4	16	≤0.25	>64	8
Ceftazidime	82.1	5.3	12.6	4	32	≤0.25	>32	4
Ceftazidime with NXL104	ND	ND	ND	2	8	≤0.06	>16	ND
Ciprofloxacin	71.3	8.3	20.4	0.25	8	≤0.06	>16	8
Colistin	91.1	8.1	0.9	2	2	≤0.06	8	72
Doripenem	85.1	NA	14.9	0.5	8	≤0.03	>32	8
Gentamicin	74.5	10.9	14.7	2	16	≤0.5	>32	8
Levofloxacin	65.3	10.9	23.8	1	16	≤0.06	>32	8
Meropenem	88.1	5.1	6.8	0.5	8	≤0.03	>32	16
Piperacillin-tazobactam	90.9	NA	9.1	4	64	≤1	>512	32

^a S = susceptible, I = intermediate, R = resistant. Breakpoint interpretation: amikacin S ≤ 16 µg/ml, I = 32 µg/ml, R ≥ 64 µg/ml; cefepime S ≤ 8 µg/ml, I = 16 µg/ml, R ≥ 32 µg/ml; ceftazidime S ≤ 8 µg/ml, I = 16 µg/ml, R ≥ 32 µg/ml; ciprofloxacin S ≤ 1 µg/ml, I = 2 µg/ml, R ≥ 4 µg/ml; colistin S ≤ 2 µg/ml, I = 4 µg/ml, R ≥ 8 µg/ml; doripenem S ≤ 2 µg/ml (FDA defined); gentamicin S ≤ 4 µg/ml, I = 8 µg/ml, R ≥ 16 µg/ml; levofloxacin S ≤ 2 µg/ml, I = 4 µg/ml, R ≥ 8 µg/ml; meropenem S ≤ 4 µg/ml, I = 8 µg/ml, R ≥ 16 µg/ml; piperacillin-tazobactam S ≤ 64/4 µg/ml, R ≥ 128/4 µg/ml. ND = breakpoints not defined; NA = not applicable, no breakpoints defined for intermediate susceptibility.

penem). Colistin (polymyxin E) was not used in the classification of MDR isolates.

In total, 470 *P. aeruginosa* isolates were obtained as a part of CANWARD in 2009 (specimen sources: respiratory [58.1%], blood [22.3%], wound [14.9%], and urine [4.7%]; ward type: medical ward [30.2%], intensive care unit [25.5%], clinic/office [25.3%], emergency room [11.9%], and surgical ward [7.0%]). Antimicrobial susceptibility data for the isolates are presented in Table 1. Addition of NXL104 to ceftazidime resulted in the lowering of ceftazidime MIC values (Fig. 1). The percentages

of *P. aeruginosa* isolates with a ceftazidime MIC of ≤8 µg/ml (CLSI ceftazidime susceptibility breakpoint) with and without NXL104 were 94.3% (443/470 isolates) and 82.1% (386/470 isolates), respectively. Of 10 ceftazidime-susceptible *P. aeruginosa* isolates tested versus NXL104 in the absence of ceftazidime, all had an NXL104 MIC of ≥32 µg/ml, indicating the lack of intrinsic antipseudomonal activity inherent to this compound.

Twenty-five *P. aeruginosa* isolates (5.3%) were MDR. The activity of ceftazidime in combination with NXL104 versus *P.*

FIG. 1. Number of *P. aeruginosa* isolates at a given ceftazidime MIC with and without NXL104.

TABLE 2. *In vitro* activity of ceftazidime in combination with NXL104 versus *P. aeruginosa* clinical isolates resistant to various antipseudomonal antimicrobials

<i>P. aeruginosa</i> (no. of isolates)	No. of isolates (cumulative % of all isolates tested) with indicated MIC ($\mu\text{g/ml}$)								Total
	≤ 0.25	0.5	1	2	4	8	16	>16	
All isolates (470)	4 (0.9)	18 (4.7)	57 (16.8)	242 (68.3)	82 (85.7)	40 (94.3)	17 (97.9)	10 (100.0)	470
Amikacin resistant (29)	1 (3.4)	2 (10.3)	1 (13.8)	9 (44.8)	5 (62.1)	6 (82.8)	2 (89.7)	3 (100.0)	29
Cefepime resistant (29)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (13.8)	10 (48.3)	6 (69.0)	9 (100.0)	29
Ceftazidime resistant (59)	0 (0.0)	1 (1.7)	0 (1.7)	6 (11.9)	15 (37.3)	17 (66.1)	10 (83.1)	10 (100.0)	59
Ciprofloxacin resistant (96)	1 (1.0)	3 (4.2)	10 (14.6)	28 (43.8)	23 (67.7)	14 (82.3)	9 (91.7)	8 (100.0)	96
Doripenem resistant (70)	0 (0.0)	0 (0.0)	4 (5.7)	14 (25.7)	21 (55.7)	18 (81.4)	7 (91.4)	6 (100.0)	70
Gentamicin resistant (69)	1 (1.4)	3 (5.8)	8 (17.4)	23 (50.7)	15 (72.5)	10 (87.0)	4 (92.8)	5 (100.0)	69
Levofloxacin resistant (112)	1 (0.9)	4 (4.5)	10 (13.4)	39 (48.2)	30 (75.0)	13 (86.6)	8 (93.8)	7 (100.0)	112
Meropenem resistant (32)	0 (0.0)	0 (0.0)	1 (3.1)	0 (3.1)	7 (25.0)	12 (62.5)	6 (81.3)	6 (100.0)	32
Piperacillin-tazobactam resistant (43)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.7)	11 (30.2)	14 (62.8)	8 (81.4)	8 (100.0)	43
MDR isolates (25)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (24.0)	9 (60.0)	5 (80.0)	5 (100.0)	25

aeruginosa isolates resistant to various antipseudomonal antimicrobials (including MDR isolates) is presented in Table 2. The percentages of MDR isolates with a ceftazidime MIC of $\leq 8 \mu\text{g/ml}$ with and without NXL104 were 60% and 4%, respectively (Tables 1 and 2).

To date, there have been few studies evaluating the *in vitro* activity of ceftazidime in combination with NXL104 versus *P. aeruginosa*. Mushtaq et al. investigated the *in vitro* activity of ceftazidime with NXL104 versus a small number of *P. aeruginosa* laboratory and clinical isolates (12). When testing *P. aeruginosa* laboratory isolates with a fully derepressed AmpC, these investigators demonstrated a drop in the ceftazidime MIC from $\geq 64 \mu\text{g/ml}$ to $\leq 8 \mu\text{g/ml}$ with the addition of NXL104. Further, of 17 clinical isolates with a ceftazidime MIC of $\geq 8 \mu\text{g/ml}$, 11 demonstrated at least a 4-fold reduction in MIC with the addition of NXL104 (12). Sahm et al. assessed the *in vitro* activity of ceftazidime in combination with NXL104 versus a random selection of 300 *P. aeruginosa* clinical isolates collected across the United States, Europe, and Asia (16). Addition of NXL104 to ceftazidime resulted in at least a 4-fold reduction in the ceftazidime MIC₉₀ (16). These data are consistent with the results presented here.

In summary, when tested against 470 *P. aeruginosa* clinical isolates, addition of NXL104 to ceftazidime lowered the ceftazidime MIC by 2- to 4-fold (1 or 2 dilutions). Sixty percent of the MDR *P. aeruginosa* isolates evaluated had a MIC of $\leq 8 \mu\text{g/ml}$ versus ceftazidime in combination with NXL104. These data suggest that the ceftazidime-NXL104 combination may prove useful in the treatment of many infections caused by *P. aeruginosa*.

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