ASM 2010
C-131

Assessment of NXL104 Disk Content to Be Used to Test Ceftaroline in Combination

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Abstract

Background: Ceftaroline (CPT) is a broad-spectrum cephalosporin with activity against Enterobacteriaceae (ENT) and many Gram-positive (GP) pathogens, including methicillin-resistant *S. aureus* (MRSA). NXL104 is a potent inhibitor of AmpC, ESBL, and KPC β-lactamases (βL). We evaluated NXL104 disk contents with CPT to optimize correlation with MIC results for CPT combined with NXL104 (CXL).

Methods: CPT disk content is established (30 µg), quality control limits are known, and PK/PD data indicate a CPT MIC susceptibility (S) breakpoint of ≤2 µg/mL. Using this baseline data, NXL104 content was optimized (60, 30, 15, 10, 5 µg) to achieve comparable accuracy in categorizing S to CXL. Analysis of results targeted maximum categorical agreement for candidate S and resistance (R) CPT breakpoints of ≤2 and ≥8 µg/mL, respectively. 151 strains, including 30 GP (*S. pneumoniae* [SPN; 10], MRSA [15], and *E. faecalis* [EF; 5]), 31 *P. aeruginosa* (PSA), 9 *A. baumannii* (ACB), and 81 ENT (10 species) producing various broad-spectrum βL (ESBL, serine carbapenemases, plasmidic AmpC, etc), were tested against CPT and CXL by broth microdilution and disk diffusion methods according to CLSI documents M07-A8 and M02-A10.

Results: MRSA, SPN, and wild-type ENT were very S to CPT and CXL, while PSA and ACB had higher MICs for both compounds. βL-producing ENT exhibited high CPT MICs and very low CXL MICs. Best categorical agreement was observed with disk breakpoints (R/S) of ≤16/≥20 mm and 30/10-µg (0.0% very major [VM], 1.9% major [MA], and 9.6% minor [MI] error rates) and 30/15-µg disks (0.0% VM, 0.7% MA, and 10.6% MI). PSA, ACB, and EF (nonindicated species) were responsible for all errors observed with CXL 30/10-µg, 30/15-µg, and 30/30-µg disks. Isolates of CPT-indicated species (106 strains) were analyzed separately, without error (see Figure 2b).

Conclusions: Regression analysis of the candidate CXL disk contents favored 30/10-µg and 30/15-µg disks to discriminate between R and S organism populations. The likelihood of resistant isolates by MIC being miscategorized as intermediate by disk was noted with 30/30- and 30/60-µg disks.

Introduction

Ceftaroline, the active form of the prodrug ceftaroline fosamil, is a broad-spectrum cephalosporin exhibiting bactericidal activity against resistant Gram-positive pathogens, including *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA), and commonly occurring Gram-negative pathogens. When combined with NXL104, a potent non-β-lactam β-lactamase inhibitor, the spectrum of activity of ceftaroline is expanded against strains producing class A β-lactamases, such as TEM, SHV, CTX-M, and KPC enzymes, class C cephalosporinases, and several class D oxacillinases with narrow or extended spectrum of activity.

The ceftaroline disk content (30 µg) has been established in previous investigations. Quality control limits have also been determined, and PK/PD data indicate a proposed ceftaroline MIC susceptibility breakpoint of approximately 2 µg/mL. Using this baseline data, NXL104 disk content was optimized (30, 15, and 10 µg) to achieve comparable accuracy among standardized methods in categorizing susceptibility to ceftaroline/NXL104.

Methods

Organisms: A total of 151 strains, including 30 Gram-positive organisms (*S. pneumoniae* [10], MRSA [15], and *Enterococcus faecalis* [5]), 31 *Pseudomonas aeruginosa*, 9 *Acinetobacter baumannii*, and 81 Enterobacteriaceae (10 species) producing various extended-spectrum β-lactamase (ESBL, serine carbapenemases, plasmidic AmpC, etc) were evaluated.

Susceptibility Testing: All organisms were tested against ceftaroline and ceftaroline combined with NXL104 (NXL104 at fixed concentration of 4 µg/mL) by broth microdilution and disk diffusion methods according to Clinical and Laboratory Standards Institute (CLSI) documents M07-A8 and M02-A10, respectively. The ceftaroline disk content has already been established at 30 µg. In the present study, the following ceftaroline/NXL104 disk contents were evaluated: 30/30 µg, 30/15 µg, and 30/10 µg. Analysis of results targeted maximum categorical agreement for candidate ceftaroline susceptibility and resistance breakpoints of ≤2 and ≥8 µg/mL, respectively.

Results

- Best categorical intermethod agreement was observed with disk diffusion method resistance and susceptibility breakpoints of ≤16 mm and ≥20 mm, respectively, and the following disk contents: 30/10-µg (0.0% very major, 1.9% major, and 9.6% minor error rates) and 30/15-µg disks (0.0% very major, 0.7% major, and 10.6% minor error rates; Table 1 and Figures 1a and 1b)
- A good correlation between methods was also obtained with the 30/30-µg disk, with a slight tendency toward higher inhibition zones. Additionally, there was an increased probability of isolates resistant by MIC being considered intermediate by disk (Table 1 and Figure 1c)
- *P. aeruginosa, A. baumannii,* and *E. faecalis* (ie, non-indicated species) were responsible for all errors observed with ceftaroline/NXL104 30/10-µg, 30/15-µg, and 30/30-µg disks (Table 1 and Figures 1 and 2)
- Isolates of ceftaroline-indicated species (106 strains) were analyzed separately, without documented categorical error (Figures 2a, 2b, and 2c)

Table 1. Summary of Error Rates According to Disk Content and Organism Group

Disk content (ceftaroline/NXL104)	Organisms	Error rate (%)		
		Very Major	Major	Minor
30 / 10 µg	All	0.0	0.7	8.6
30 / 10 µg	Indicated species[a]	0.0	0.0	0.0
30 / 15 µg	All	0.0	0.7	10.6
30 / 15 µg	Indicated species[a]	0.0	0.0	0.0
30 / 30 µg	All	0.0	0.0	10.6
30 / 30 µg	Indicated species[a]	0.0	0.0	0.0

a. Includes 106 organisms: *S. aureus* (n = 15), *S. pneumoniae* (n = 10), *Klebsiella* spp. (n = 31), *E. coli* (n = 27), *Enterobacter* spp. (n = 10), *S. marcescens* (n = 6), *Citrobacter* spp. (n = 4), *M. morganii* (n = 2), and *P. mirabilis* (n = 1).

Figure 1. Ceftaroline/NXL104 (fixed 4 µg/mL) MIC vs Ceftaroline/NXL104 Disks 30/10 µg (1a), 30/15 µg (1b), and 30/30 µg (1c) – All Strains

Figure 1a

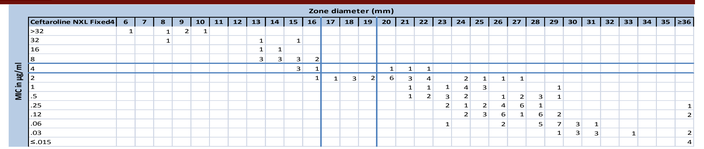


Figure 1b

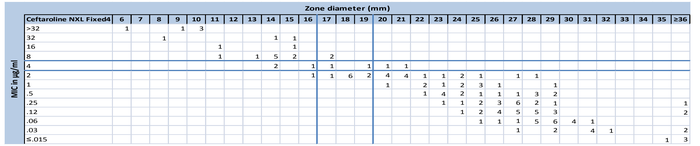


Figure 1c

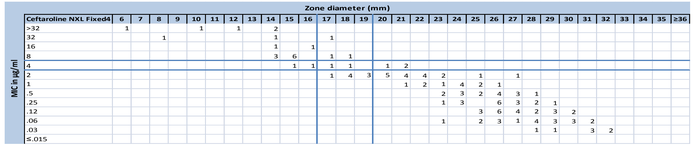


Figure 2. Ceftaroline/NXL104 (fixed 4 µg/mL) MIC vs Ceftaroline/NXL104 Disks 30/10 µg (2a), 30/15 µg (2b), and 30/30 µg (2c) – Indicated Species Only

Figure 2a

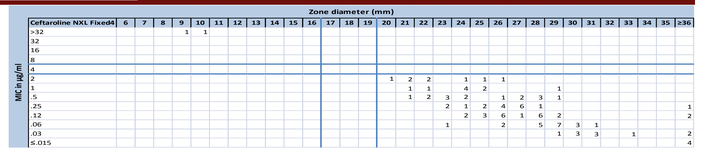


Figure 2b

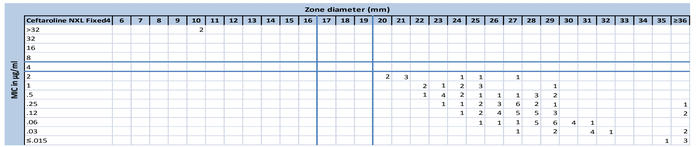
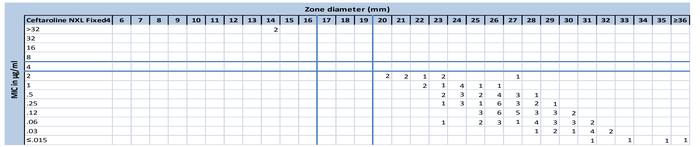


Figure 2c



Conclusions

- Regression analysis of the candidate ceftaroline/NXL104 disks favored the use of 30/10-µg and 30/15-µg disks to discriminate between resistant and susceptible organism populations
- Complete categorical agreement was observed with ceftaroline/NXL104 (fixed 4 µg/mL) MIC values and the 30/10-µg, 30/15-µg, or 30/30-µg disk results when only bacterial isolates of indicated species were analyzed

References

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Acknowledgment

Supported by Forest Laboratories, Inc.