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Interim Susceptibility Testing for Ceftaroline, a Novel MRSA-Active Cephalosporin: Selecting Potent Surrogate β-Lactam Markers to Predict CPT Activity against Clinically Indicated Species

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Abstract

Background: Ceftaroline (CPT), the active form of CPT fosamil, has *in vitro* activity against resistant Gram-positive organisms, including MRSA and multidrug-resistant (R) *S. pneumoniae* (SPN). CPT was FDA-approved in late 2010 for the indications of community-acquired bacterial pneumonia and acute bacterial skin/skin structure infections. For newly marketed antimicrobials, few susceptibility (S) test methods are initially available and commercial products (especially automated systems) may not be FDA-approved for more than a year. Interim strategies include testing by agar diffusion methods (disk and Etest) or applying a surrogate test agent that affords high predictive accuracy, especially for MRSA.

Methods: To identify surrogate β-lactams to predict CPT-S, 14,902 USA organisms (3954 SPN; 769 *H. influenzae* [HI]; 8619 *S. aureus* [SA; 53.6% MRSA]; and 1560 indicated Enterobacteriaceae [ENT]) were S tested by CLSI broth microdilution and interpretations, except for CPT (FDA package insert).

Results: For SPN, S to ceftriaxone (CRO) or cefepime (CPM) or amoxicillin/clavulanate (A/C) would accurately predict CPT-S at rates of 99.97, 99.97 and 99.94%, respectively (87.17% of CRO-R pneumococci were CPT-S). HI S to CPT would be predicted at high rates (99.87%) using CRO or CPM or ceftazidime S results. For SA, CPT-S was best predicted by carbapenems (imipenem [IMP] at 99.86%; meropenem [MER] at 99.89%) and IMP- or MER-S or Intermediate (I; MIC, ≤8 μg/ml) correlated with CPT-S with 99.75 - 99.83% accuracy. CRO was the best surrogate for CPT when testing ENT (95.89%).

Conclusions: CPT can accurately be S tested using a β-lactam surrogate strategy for commercial systems generating MIC results (Vitek® 2, Phoenix™, MicroScan®). Accuracy among SPN (99.94-99.97%), HI (99.87%), and SA (99.75-99.89%) was highly acceptable. Among SA 73.99-79.36% of IMP-or MER-R strains remained CPT-S, further minimizing predictive risk and allowing CPT clinical use with local *in vitro* test guidance.

Introduction

Ceftaroline (CPT), the active form of CPT fosamil, is a broad-spectrum cephalosporin with a uniquely high binding affinity for the altered penicillin-binding protein (PBP2a) responsible for methicillin resistance among staphylococci. Unlike other cephalosporins that are inactive against methicillin-resistant *Staphylococcus aureus* (MRSA), ceftaroline has demonstrated *in vitro* potency and clinical success against this important pathogen and has a clinical indication for use in acute bacterial skin and skin structure infections as well as community-acquired bacterial pneumonia (CABP; not MRSA). Therefore, the use of oxacillin and/or cefoxitin test results to predict ceftaroline resistance or susceptibility among other β-lactams does not apply and direct testing of this new cephalosporin would be desirable to predict clinical success per criteria approved in the United States Food and Drug Administration (USA-FDA) product package insert. Those USA-FDA staphylococcal susceptibility criteria are a ceftaroline MIC at ≤1 μg/ml and a zone diameter of ≥24 mm when using methods published by the Clinical and Laboratory Standards Institute (CLSI). Non-susceptible results (MIC, ≥2 μg/ml) have not been characterized as either intermediate or resistant due to limited clinical experience with infections caused by staphylococci having those MIC levels.

Similarly, ceftaroline exhibits a high binding to altered PBPs associated with β-lactam MIC elevations in streptococci, particularly *Streptococcus pneumoniae*. This resulting potency advantage compared to ceftriaxone expands the spectrum of ceftaroline against this CABP pathogen and translates to high clinical success rates.

As the vast majority (>80%) of clinical microbiology laboratories do not use reference/standardized CLSI methods, alternative strategies for testing newly released antimicrobials must be developed due to long term delays in the development and USA-FDA approval of commercial susceptibility testing products (Vitek®, Vitek® 2, BD Phoenix™, MicroScan®, Sensititre®). As some antimicrobials may present immediate therapeutic advantages, one strategy is to test a surrogate agent (usually in the same class) as a predictor of susceptibility and/or resistance. This testing option has been most recently applied to doripenem, but has also been used for other β-lactams (cefotetan, cefpodoxime), and by the CLSI in Table 1 of document M100-S22. The most difficult obstacle for ceftaroline has been to select an appropriate antimicrobial class (β-lactam) agent when testing MRSA strains, where no other commercially available β-lactam has demonstrated clear *in vitro* and clinical utility. However, some carbapenems have shown measurable potencies versus MRSA that may be usable, as would advanced-spectrum cephalosporins (ceftriaxone, ceftazidime or cefepime) when testing *Streptococcus* spp. This study investigated the optimal use of candidate β-lactams as surrogate predictors of ceftaroline activity/susceptibility, allowing the earliest guided clinical use in medical centers having FDA-approved commercial susceptibility testing systems reporting quantitative MIC values or category interpretations using the CLSI and USA-FDA breakpoint criteria.

Methods

The organisms tested in the Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) Surveillance Program from 2008-2010 were analyzed to select a surrogate marker agent for ceftaroline. The species selected were: 3954 *S. pneumoniae* (2008-2010); 769 *Haemophilus influenzae* (2010 only); 8619 *S. aureus* (2008-2010; 53.6% MRSA); and 1560 indicated Enterobacteriaceae (2010 only, *E. coli* and *Klebsiella* spp), for a total of 14,902 strains, all tested by the CLSI M07-A9 method in a GLP facility (JMI Laboratories, North Liberty, Iowa). Concurrent quality control (QC) strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and 29213, *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619 were used, and all QC results were within published ranges for ceftaroline and candidate surrogate β-lactams.

Analysis focused on the identification of surrogate agents to predict ceftaroline susceptibility minimizing, where possible, false-susceptibility to ≤1.5% and false-intermediate rates to ≤5%. Comparisons used published breakpoint criteria for each agent. With the exception of testing the Enterobacteriaceae, ceftaroline only has susceptible and non-susceptible criteria precluding total cross-resistance calculations. By using the most potent β-lactam surrogate agents against each pathogen, the interpretive error was found to be far below the listed target accuracy limits above (see Tables and Figures).

Results

Surrogate Candidates for Staphylococci

– Carbapenems (imipenem and meropenem) demonstrated measurable activities against the tested MRSA (53.6% of *S. aureus* sample), having MIC values ranging from ≤0.12 to >8 μg/ml for both agents. This high carbapenem activity against MRSA and all *S. aureus* made them unique surrogate candidates among β-lactams and demonstrated a linear relationship to ceftaroline MIC results (Figures 1 and 2)

– For imipenem used as a surrogate for ceftaroline susceptibility (Figure 1), an MIC of ≤4 μg/ml to imipenem was 99.86% accurate in predicting a susceptible ceftaroline MIC value (≤1 μg/ml). By also adding imipenem MIC results at 8 μg/ml (eg. ≤8 μg/ml for imipenem = ceftaroline-susceptible), the accuracy rate only decreased to 99.75%. The accuracy of ceftaroline-susceptible values predicted by meropenem MIC results at ≤4 and ≤8 μg/ml was 99.89 and 99.83%, respectively (Figure 2)

– Among imipenem-and meropenem-non-susceptible *S. aureus* tested (MICs, ≥8 μg/ml), 73.99 and 79.36% of isolates (all MRSA) remained susceptible to ceftaroline

Surrogate Candidates for *S. pneumoniae*

– Only the most active commercially available β-lactams would be possible surrogates for ceftaroline susceptibility and their accuracy is shown in Table 1. The accuracy rates of ceftriaxone (99.97%) and cefepime (99.97%) were best to predict ceftaroline-susceptible *S. pneumoniae*; however, amoxicillin/clavulanate (99.94%) could also be used with confidence. Note that utilizing amoxicillin/clavulanate susceptibility results significantly underestimated coverage of ceftaroline when compared to using ceftriaxone (Figure 3 and Table 1)

– Also no ceftriaxone-intermediate strains (336 isolates) were ceftaroline-non-susceptible (Table 1); therefore a ceftriaxone MIC at ≤2 μg/ml predicted ceftaroline susceptibility with an accuracy of nearly 100.0%

Surrogate Candidates for *H. influenzae*

– All three agents (ceftriaxone, cefepime, and ceftazidime) performed well (99.87% accuracy) and can be used with confidence as surrogate agents for ceftaroline susceptibility versus *H. influenzae*; even with the very low USA-FDA ceftaroline breakpoint at ≤0.12 μg/ml, compared to the other cephalosporins (≤2 μg/ml as susceptible; see Table 2)

Surrogate Candidates for Indicated Enterobacteriaceae

– A total of 1560 *E.coli* and *Klebsiella* spp. isolated in 2010 were used to assess the accuracy of using ceftriaxone (Figure 4) and other cephalosporins to predict ceftaroline susceptibility at ≤0.5 μg/ml. Using ceftriaxone to predict ceftaroline-susceptible strains showed a 95.89% accuracy with 2.09 and 2.02% minor and very major errors, respectively. If the ceftaroline-susceptible breakpoint was adjusted to ≤1 μg/ml (2 μg/ml as intermediate), like that used for *S. aureus*, the accuracy rate would be 97.98% for ceftriaxone with only 1.37% very major error (acceptable), see Figure 4.

Table 1. Susceptibility Category Comparisons of Ceftaroline with Three Broad-spectrum β-lactams (Ceftriaxone, Cefepime, Amoxicillin/clavulanate) when Tested against 3954 *S. pneumoniae* from the USA

		No. ceftaroline MICs by category (μg/ml):		
		Susceptible	Non-Susceptible	
Surrogate candidate	CLSI category (MIC)	(≤0.25)	(0.5)	(≥1)
Ceftriaxone	Susceptible (≤1)	3532[a]	1[a]	0
	Intermediate (2)	336	0	0
	Resistant (≥4)	31	54	0
Cefepime	Susceptible (≤1)	3537[b]	1[b]	0
	Intermediate (2)	354	29	0
	Resistant (≥4)	8	25	0
Amoxicillin/clavulanate	Susceptible (≤1)	3283[c]	2[c]	0
	Intermediate (2)	107	4	0
	Resistant (≥4)	509	49	0

a. Accuracy of ceftriaxone susceptibility results to predict ceftaroline susceptibility at 3532/3533 (99.97%); among the 421 ceftriaxone-non-susceptible pneumococcal strains, 87.17% were ceftaroline-susceptible.
b. Accuracy at 99.97% (3537/3538).
c. Accuracy at 99.94% (3283/3285).

Table 2. Susceptibility Category Comparisons of Ceftaroline with Three Cephalosporins (Ceftriaxone, Cefepime, Ceftazidime) when Tested against 769 *H. influenzae* from the USA

		No. ceftaroline MICs by category (μg/ml):	
		Susceptible (≤0.12)	Non-susceptible (≥0.25)
Surrogate candidate	CLSI category (MIC)		
Ceftriaxone	Susceptible (≤2)	768[a]	1[a]
	Non-susceptible (≥4)	0	0
Cefepime	Susceptible (≤2)	768[a]	1[a]
	Non-susceptible (≥4)	0	0
Ceftazidime	Susceptible (≤2)	768[a]	1[a]
	Non-susceptible (≥4)	0	0

a. Accuracy of ceftriaxone, cefepime and ceftazidime susceptibility results to predict ceftaroline susceptibility was at 768/769 (99.87%).

Figure 1. Scattergram of Ceftaroline MIC Values Compared to Imipenem MIC Results when Tested against 8619 *S. aureus* (4624 or 53.6% were MRSA) from the USA. Solid Bolded Horizontal (USA-FDA) and Vertical (CLSI) Lines Indicate Breakpoints for Each Agent

Ceftaroline MIC (μg/ml) \ Imipenem MIC (μg/ml)	≤0.12	0.25	0.5	1	2	4	8	>8
≥4							9	161
2	3	1	2	2	1	2		
1	120	328	425	306	226	189	159	435
0.5	1210	586	399	176	64	23	15	23
0.25	3388	25	9	5	3			
0.12	295							
≤0.06	29							

Figure 2. Scattergram of Ceftaroline MIC Values Compared to Meropenem MIC Results when Tested against 8619 *S. aureus* (4624 or 53.6% were MRSA) from the USA. Solid Bolded Horizontal (USA-FDA) and Vertical (CLSI) Lines Indicate Breakpoints for Each Agent

Ceftaroline MIC (μg/ml) \ Meropenem MIC (μg/ml)	≤0.12	0.25	0.5	1	2	4	8	>8
≥4								
2			1	2	3	2	5	168
1	3	9	47	264	613	328	311	613
0.5	277	165	416	771	579	184	71	33
0.25	3212	166	20	14	13	3	2	
0.12	287	7	1					
≤0.06	28	1						

Figure 3. Scattergram of Ceftaroline MIC Values Compared to Ceftriaxone MICs when Testing 3954 *S. pneumoniae* Isolates from the USA. Only 1.39% of Pneumococci were Ceftaroline Non-susceptible and No MIC was Observed at >0.5 μg/ml. Solid Bolded Horizontal (USA-FDA) and Vertical (CLSI) Lines Indicate Breakpoints for Each Agent

Ceftaroline MIC (μg/ml) \ Ceftriaxone MIC (μg/ml)	≤0.25	0.5	1	2	4	8	>8
≥1							
0.5		1			17	37	
0.25	1	1	63	218	19	8	
0.12	7	53	327	116	1	1	
0.06	186	122	28	1			
≤0.03	2720	23	1	1	1	1	

Figure 4. Scattergram Comparing Ceftaroline and Ceftriaxone MIC Values when Testing 1560 Indicated Enterobacteriaceae (*E. coli* and *Klebsiella* spp.). Two Ceftaroline Breakpoints are Shown, 1.) USA-FDA Product Package Insert (Solid Bolded Horizontal Lines) and 2.) ≤1 μg/ml as Susceptible, Like that Applied to Staphylococci (Broken Horizontal Line)

Ceftaroline MIC (μg/ml) \ Ceftriaxone MIC (μg/ml)	≤0.06	0.12	0.25	0.5	1	2	4	8	>8
≥16		2		2	2	1	7	8	124
8		1	4		2		6	7	3
4		1	3	2			2	1	3
2	4	3	1		1	2	3	2	
1	4	13	9	2	1		2		
0.5	39	25	23	3	1	1	1		
0.25	103	64	7		1				
0.12	309	55	6		1				
0.06	536	23							
≤0.03	134								

Conclusions

- This interim ceftaroline susceptibility testing strategy of using potent β-lactams as surrogate agents was examined using 14,902 recent clinical isolates tested by reference CLSI broth microdilution methods and published breakpoint concentrations (CLSI and USA-FDA)
- High-level accuracy for predicting ceftaroline susceptibility was observed as follows: for *S. aureus* use either imipenem or meropenem at a susceptible MIC of ≤8 μg/ml (99.75-99.83% accurate); for *S. pneumoniae* use ceftriaxone at a susceptible MIC of ≤2 μg/ml or at ≤1 μg/ml (≥99.97% accurate); for *H. influenzae* use ceftriaxone or cefepime or ceftazidime at a susceptible MIC of ≤2 μg/ml (99.87% accurate); and for indicated *E. coli* and *Klebsiella* spp. use ceftriaxone at a susceptible MIC of ≤1 μg/ml (95.89% accurate)
- Like previous uses of surrogate agents within the β-lactam antimicrobial class, these qualified uses of carbapenems (*S. aureus*) and "third-or fourth-generation" cephalosporins (*S. pneumoniae, H. influenzae,* and indicated Enterobacteriaceae) for ceftaroline testing offers immediate applications to existing susceptibility testing results generated by commercial systems producing quantitative (MICs) or categorical/qualitative results.

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