Research Article

Activity of Ceftaroline against Aerobic Gram-Positive and Gram-Negative Pathogens: Effect of Test Method Variability

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Ceftaroline is a new cephalosporin with bactericidal activity against methicillin-resistant S. aureus (MRSA) as well as gram-negative pathogens. Variations of in vitro test conditions were found to affect ceftaroline activity, with 5% NaCl inhibiting growth and/or reducing the minimum inhibitory concentrations (MICs) for E. coli, K. pneumoniae, M. catarrhalis, H. influenzae, and streptococci, while an inoculum of 10⁶ CFU/mL raised MICs of some E. coli, K. pneumoniae, and M. catarrhalis strains.

1. Introduction

The emergence of MRSA has spurred the development of alternative therapies such as daptomycin, linezolid, and quinupristin-dalfopristin, which are not active against gram-negative pathogens and require combination therapy. Ceftaroline is a new, parenteral, broad-spectrum cephalosporin with bactericidal activity against MRSA, including vancomycin-intermediate (VISA) strains, and multidrug-resistant Streptococcus pneumoniae (MDRSP); it is also active against common gram-negative pathogens and can therefore be used as monotherapy for mixed infections [1–6]. Since alterations of in vitro test conditions can potentially affect susceptibility results, we evaluated the effects of 15 variations to the standard test conditions as specified by the Clinical and Laboratory Standards Institute (CLSI) guidelines [7, 8] on the minimum inhibitory concentrations (MICs) of ceftaroline against 30 isolates representing 10 species of clinically important, commonly encountered organisms.

2. Materials and Methods

2.1. Standard Method. The CLSI reference broth microdilution method (CLSI 2006, 2009) uses cation-adjusted Mueller Hinton broth (CAMHB) (Difco, BD; Sparks, Md, USA), which has a calcium concentration of 25 mg/L, a magnesium concentration of 12.5 mg/L, and a pH of 7.3 ± 0.1. The standard inoculum is 5 × 10⁵ colony-forming units (CFUs)/mL for broth microdilution testing and 10⁴ CFU/spot for agar dilution tests.

2.2. Test Variables. Modifications of standard test conditions included adjusting the Ca²⁺ content of CAMHB to 50 mg/L Ca, addition of NaCl to 5%, adjusting the broth to pH 6 and pH 8, and using inocula of 10⁴ and 10⁶ colony-forming units (CFUs)/mL. Other variations to the standard medium were the addition of 10% and 50% pooled human serum (Sigma; St. Louis, Mo, USA), the addition of lysed horse blood to 2.5% (LHB) (Hardy Diagnostics, Inc. Santa Maria, Calif, USA), and using Haemophilus test medium (HTM) broth. While MIC panels were incubated at 35°C in ambient conditions, for comparative purposes, additional tests in CAMHB were incubated in the anaerobic chamber or in 5% CO₂.

2.3. MIC Test Panel Preparation. Ninety-six-well panels were prepared with twice the final concentration of ceftaroline (50 μL/well) using the Quick-Spense Ile apparatus (Sandy Springs Instruments; Germantown, Md, USA) and stored at −70°C until used. Addition of 50 μL of the organism inocula to the wells reduced the final ceftaroline concentration to
Table 1: List of organisms used in the study.

| Organism          | RMA number | Specimen source       | Date isolated | Comments                |
|-------------------|------------|-----------------------|---------------|-------------------------|
| E. coli           | 19090      | Blood                 | 3/7/2007      | Ampicillin = 4 μg/mL    |
|                   | 19091      | Primary infection site| 3/1/2007      | Ampicillin ≥32 μg/mL    |
| K. pneumoniae     | 19092      | Blood                 | 6/6/2007      | Ampicillin = 16 μg/mL   |
|                   | 19093      | Blood                 | 6/29/2007     | Ampicillin = 32 μg/mL   |
| H. influenzae     | 16081      | Respiratory-sinus     | 12/31/2003    | β-Lactamase-negative    |
|                   | 18520      | Respiratory-sinus     | 12/23/2005    | β-Lactamase-positive    |
| M. catarrhalis    | 11940      | Respiratory-sinus     | 6/14/2000     |                         |
|                   | 14032      | Respiratory-sinus     | 5/22/2002     |                         |
|                   | 18861      | Respiratory-sputum    | 1/31/2007     |                         |
| S. aureus         | 18488      | Chest infection site  | 2/11/2005     | Methicillin-S           |
|                   | 18401      | Blood                 | 8/16/2005     | Methicillin-S           |
|                   | 18483      | Head abscess          | 10/15/2005    | Methicillin-R           |
|                   | 18504      | Primary infection site| 11/18/2005    | Methicillin-R           |
|                   | 18526      | Blood                 | 10/24/2005    | Methicillin-R           |
| E. faecalis       | 18284      | Foot infection site   | 3/24/2005     |                         |
|                   | 18877      | Blood                 | 4/10/2007     |                         |
| S. pyogenes       | 17018      | Diabetic foot infection site | 1/22/2003 |                         |
|                   | 17019      | Diabetic foot infection site | 10/22/2002 |                         |
|                   | 19047      | Abdominal lesion      | 10/26/2007    | Clindamycin-R           |
| S. pneumoniae     | 19094      | Ear                   | 10/29/2007    | Penicillin-S            |
|                   | 19095      | Eye                   | 1/9/2007      | Penicillin-S            |
|                   | 13345      | Nasopharynx           | 11/14/2001    | Penicillin = 8 μg/mL    |
|                   | 13385      | Nasopharynx           | 12/4/2001     | Penicillin = 8 μg/mL    |
|                   | 18876      | Eye                   | 1/2/2007      | Penicillin = 8 μg/mL    |

RMA: R.M. Alden (culture collection).
ATCC: American Type Culture Collection.

the desired level of 0.008 to 8 μg/mL. Some of the organisms did not achieve a ceftaroline MIC endpoint, and further dilutions were prepared to 0.001 μg/mL for retesting some of those isolates.

2.4. Agar Dilution Test Media. Agar dilution MICs were determined on unsupplemented Mueller Hinton agar (MHA) (Difco), with 5% LHB, and on HTM with 1.5% agar (HTMA). Serial twofold dilutions of ceftaroline were added to molten agar deeps to prepare the plates for use on the same day. Concentrations of ceftaroline ranged from 0.008 to 8 μg/mL. Drug-free growth control plates were included (CLSI, 2006).

2.5. Test Organisms. All 30 strains tested were recent clinical isolates and American Type Culture Collection (ATCC) quality control (QC) strains, which included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, and *Haemophilus influenzae* ATCC 49247. Details about the clinical isolates are listed in Table 1. Clinical isolates were selected based on previously demonstrated resistance patterns. The isolates were stored in 20% skim milk at −70°C and were taken from frozen stock and transferred twice on blood or chocolate agar (Hardy Diagnostics Inc.) before testing.

2.6. Inoculum Preparation for Microbroth Dilution Tests. Standard inocula were prepared by suspending colonies from overnight cultures in 0.85% saline to equal the turbidity of the 0.5 McFarland standard and diluting it in CAMHB with the various additives at twice their final concentration, which upon addition of 50 μL of inoculum to the test panel were diluted 1:2. The 10^4 and 10^6 cfu/mL inocula were prepared by diluting the saline suspension either 10-fold more (for 10^4 cfu/mL) or 10-fold less (for 10^6 cfu/mL). The trays were inoculated with 50 μL of cell suspension for a final inoculum of ~5 × 10^8 CFU/mL which was validated by
Table 2: Ceftaroline MICs (μg/mL) in the cation-adjusted Mueller-Hinton broth with test variables and agar dilution MICs.

| Organism          | RMA number | REF | 50 mg/L | 5 | 6 | 8 | 10 | 10^4 | 10 | 50 | LHB | MHA | HTM | CO₂ | Anaerobic |
|-------------------|------------|-----|---------|---|---|---|----|------|----|----|-----|-----|-----|-----|-----------|
| E. coli           | ATCC 25922 | 0.06 | 0.06   | ≤0.008 | 0.125 | 0.06 | 0.06 | 0.125 | 0.06 | 0.125 | 0.125 | 0.125 | 0.03 | 0.06 | 0.125 | 0.25 | 0.25 |
| H. influenzae     | ATCC 49247 | 0.015 | 0.015 | ng | ng | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | n/t | 0.06 | 0.03 | ng | 0.06 | 0.125 |
| M. catarrhalis    | ATCC 11940 | 0.125 | 0.06 | ng | ng | 0.125 | 0.03 | 2 | 0.06 | 0.125 | 0.125 | 0.125 | 0.125 | 0.03 | ng | 0.06 | 0.03 | n.g. |
| S. aureus -MSSA  | ATCC 29213 | 0.125 | 0.125 | 0.125 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 |
| S. aureus -MRSA  | ATCC 18483 | 0.25 | 0.5 | 0.25 | 0.5 | 0.5 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1 |
| E. faecalis       | ATCC 29212 | 0.5 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 2 | 0.5 | 2 |
| S. pyogenes       | ATCC 17018 | 0.002 | 0.002 | ng | 0.002 | 0.002 | 0.002 | 0.004 | 0.004 | 0.002 | n/t | 0.004 | 0.004 | 0.004 | ≤0.008 | ≤0.008 | 0.015 |
| S. pneumoniae     | ATCC 49619 | 0.015 | 0.015 | ng | 0.015 | 0.008 | 0.008 | 0.015 | 0.015 | 0.008 | n/t | 0.015 | 0.015 | 0.015 | ≤0.008 | 0.015 | ≤0.008 |

ATCC: American Type Culture Collection; CFUs: colony-forming units; HTM: *Haemophilus* test medium; HTMA: HTM with 1.5% agar; LHB: laked horse blood; MHA: Mueller Hinton agar; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*; ng: no growth; nt: not tested; REF: reference method; RMA: R.M. Alden (culture collection).

All *H. influenzae* were tested in HTM; all streptococci were tested with 2.5% LHB supplementation.
quantitative subculture from the growth control well. Inoculum preparation and all testing were performed in duplicate.

2.7. Agar Dilution Testing. For agar dilution tests, the cell suspensions prepared as above were diluted 1:10 in CAMHB and applied to the agar plates using a Steers replicator device that delivered a final inoculum of 10⁶ CFU/spot.

2.8. MIC Determinations. After overnight incubation, the broth microdilution trays were examined for growth. The MIC was the lowest drug concentration that completely inhibited growth [7]. For agar dilution, the plates were incubated at 35°C overnight. The MIC was the lowest concentration that completely inhibited growth or resulted in a marked reduction of growth as compared with the drug-free control [7].

3. Results

We obtained MICs from duplicate tests under the variations shown in Table 2. In cases of discrepancy, the higher value was recorded. The ceftaroline MICs for the QC isolates (tested with the reference microbroth methods according to CLSI guidelines) were all within their acceptable ranges. Effects of variables in testing were noted where 5% NaCl inhibited growth and/or reduced MICs for E. coli and K. pneumoniae and completely inhibited the growth of M. catarrhalis, H. influenzae, and all streptococci. Using an increased inoculum of 10⁶ cfu/mL increased the MIC 5-fold for 1 of 3 E. coli strains that was also resistant to ampicillin (MIC >32 μg/mL) and 1 of 3 K. pneumoniae strains that did not appear to have any unusual resistance pattern (ampicillin MIC 32 μg/mL, ceftriaxone 0.25 μg/mL). This K. pneumoniae isolate produced the same result when retested. The higher inoculum also increased MICs 3- to 5-fold for M. catarrhalis. The addition of blood or serum to the medium enhanced M. catarrhalis growth without changing the MICs. Testing on agar, especially HTMA, produced MICs that were 1–3 dilutions lower. E. coli, K. pneumoniae, M. catarrhalis, H. influenzae, and S. pyogenes grew poorly with NaCl supplementation and at pH 6.0. An inoculum of 10⁶ CFU/mL also increased the MICs four- to six-fold for all three M. catarrhalis strains tested.

5. Conclusion

The in vitro antibacterial activity of ceftaroline was adversely affected by 5% NaCl which inhibited growth and/or reduced MICs for E. coli, K. pneumoniae, M. catarrhalis, H. influenzae, and streptococci, while an inoculum of 10⁶ CFU/mL raised MICs of some E. coli, K. pneumoniae, and M. catarrhalis strains. The other modifications tested did not adversely affect MIC results. Organisms with special growth requirements can be tested for ceftaroline susceptibility with reasonable assurance that test conditions will not affect the MIC results.

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