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# Evaluation of the Bactericidal Activity of the Novel Cephalosporin Ceftaroline (PPI-0903M) Compared to Ceftriaxone against *Streptococcus pneumoniae*

**HS SADER, G MOET, TR FRITSCHE, RN JONES**
**JMI Laboratories, North Liberty, IA**

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JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370
fax 319.665.3371
ronald-jones@jmilabs.com

## ABSTRACT

**Background**: Ceftaroline (CFT), formerly PPI-0903M, is a novel *N*-phosphono cephalosporin with potent activity against MRSA and many other Gram-positive organisms, including penicillin (PEN)-resistant (R) *S. pneumoniae* (SPN). The bactericidal action of CFT and ceftriaxone (CRO) was evaluated against PEN-R and PEN-susceptible (S) pneumococci.

**Methods**: 72 recent SPN clinical isolates, including 50 PEN-R, 11 PEN-intermediate and 11 PEN-S, had CFT and CRO MIC and MBC values determined by CLSI reference methods. MBCs were assessed by plating the entire amount of broth (100 µL) from the MIC well and those $log_2$ dilutions above onto appropriate growth media. The lowest concentration that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint. Kill-curve experiments (KC) were performed on 12 strains (10 PEN-R, 2 PEN-S) at 2X, 4X and 8X MIC. Time points included $T_0$, $T_2$, $T_4$, $T_8$ and $T_{24}$. Cidal activity was defined as a reduction of ≥3 $log_{10}$ CFU/ml.

**Results**: CFT ($MIC_{50/90}$, 0.12/0.25 µg/ml) was 8-fold more potent than CRO ($MIC_{50/90}$, 1/2 µg/ml). CFT and CRO demonstrated bactericidal activity ≤2-fold the MIC for 90.3% of tested organisms and 94.4% of strains had a MBC/MIC ratio of ≤4 for both drugs. CFT KC results are summarized in the table.

| Resistance pattern (no.) | Average $log_{10}$ CFU/ml at 4X CFT MIC (time in hours) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 8 | 24 |
| PEN-S (2) | 4.6E5 | 2.7E4 | 1.4E4 | 1.9E3 | 1.0E1 |
| PEN-R (10) | 5.6E5 | 9.2E4 | 5.7E4 | 1.0E4 | 6.1E1 |

CFT showed cidal activity at 4X and 8X MIC against all 12 strains while one strain showed re-growth at 4X CRO MIC. Cidal activity was achieved against 11 stains at 2X MIC, while 1 PEN-S strain showed re-growth at 2X MIC when tested against both cephalosporins.

**Conclusions**: CFT was highly bactericidal against SPN strains, and R to PEN does not adversely influence CFT bactericidal activity. This MRSA-active cephalosporin also possesses significant anti-SPN potency.

## INTRODUCTION

Ceftaroline (formerly PPI-0903) is a new cephalosporin with broad-spectrum antimicrobial activity and is in early clinical development. Ceftaroline is very active against many clinically important bacterial pathogens, including streptococci (ß-haemolytic, viridans group and *Streptococcus pneumoniae*), staphylococci (*Staphylococcus aureus* and coagulase-negative species), *Haemophilus influenzae, Moraxella catarrhalis* and many Enterobacteriaceae species, among others. In addition, ceftaroline has enhanced potency against gram-positive cocci, including multidrug-resistant (MDR) *S. pneumoniae* and methicillin-resistant *S. aureus* (MRSA). This compound has a high affinity for PBP2' or PBP2A and shows a potent bactericidal activity against MRSA.

Previous studies have also shown that ceftaroline retained excellent antimicrobial activity against pneumococcal isolates; including penicillin-intermediate ($MIC_{90}$, 0.06 µg/ml), penicillin-resistant ($MIC_{90}$, 0.25 µg/ml) and MDR ($MIC_{90}$, 0.25 µg/ml) strains. The present study further evaluated the bactericidal activity of ceftaroline by testing a selected collection of contemporary penicillin-susceptible, -intermediate and -resistant *S. pneumoniae* strains.

## MATERIALS AND METHODS

Bacterial Isolates:
- MIC and MBC tests: A total of 72 recent clinical strains of *S. pneumoniae* were evaluated, including 50 penicillin-resistant, 11 penicillin-intermediate and 11 penicillin-susceptible strains.
- Time kill curve experiments were performed on 12 *S. pneumoniae* strains, including 10 penicillin-resistant strains (MIC, ≥2 µg/ml) and two penicillin-susceptible strains (MIC, ≤0.06 µg/ml) tested as controls. These 12 strains were included in the MIC/MBC experiments.

Susceptibility Testing: Isolates were tested for susceptibility by reference broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria. Reagent powder of ceftaroline was provided by Cerexa Inc, while comparator agents were provided by the respective manufacturers or purchased from Sigma Aldrich Co. (St. Louis, MO). MIC panels were prepared at JMI Laboratories (North Liberty, IA) and frozen at -70°C until used. CLSI M100-S16 (2006) quality control criteria were used for comparators.

Minimum Bactericidal Concentration: MBC values were assessed for ceftaroline and ceftriaxone by plating the entire amount of broth (100 µL) from the MIC well and from those $log_2$ dilutions above the MIC for each organism onto appropriate growth media. Quantitative colony counts were performed on the starting inoculum at the time the MIC test was performed. The lowest concentration of antimicrobial agent that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint.

Time Kill Curve: Bactericidal activities of ceftaroline and ceftriaxone were evaluated by time kill curve experiments according to Moody & Knapp (2004) and NCCLS M26-A. Two samples of each strain were tested (total of 24 kill curves). The antimicrobial agents were tested at 2X, 4X and 8X MIC. Colony counts were performed at $T_0$, $T_2$, $T_4$, $T_8$ and $T_{24}$.

## RESULTS

- Ceftaroline ($MIC_{50}$, 0.12 µg/ml and $MIC_{90}$, 0.25 µg/ml) was generally eight-fold more potent than ceftriaxone ($MIC_{50}$, 1 µg/ml and $MIC_{90}$, 2 µg/ml) against the collection of 72 *S. pneumoniae* strains evaluated (Table 1).
- Ceftaroline MBC values ($MBC_{50}$, 0.12 µg/ml) were generally eight- to 16-fold lower than those for ceftriaxone ($MBC_{50}$, 2 µg/ml; Table 1).
- MBC/MIC ratios were similar for both cephalosporins tested (Table 2). Only four strains (5.6%) showed MBC/MIC ratios ≥32 for both cephalosporins, three were penicillin-resistant (6.0% of penicillin-resistant strains tested) and one was penicillin-intermediate (9.1% of penicillin-intermediate strains tested).
- Ceftaroline and ceftriaxone showed bactericidal activity by time-kill curve method (reduction of ≥3 $log_{10}$ CFU/ml of the initial inoculum) against 11 of 12 strains selected for this experiment at 2X, 4X and 8X MIC (Table 3 and Figure 1).
- One strain (064-119B, penicillin-susceptible) showed re-growth at 2X ceftaroline MIC and at 2X and 4X ceftriaxone MIC. This strain had a ceftaroline MIC of 0.015 µg/ml and ceftriaxone MIC of 0.06 µg/ml.

**Table 1.** Frequency of occurrence of MIC and MBC values for ceftaroline and ceftriaxone when tested against 72 *S. pneumoniae* strains.

| Antimicrobial concentration (µg/ml) | No. of isolates (cumulative percentage) at: MIC | | MBC | |
|---|---|---|---|---|
| | Ceftaroline | Ceftriaxone | Ceftaroline | Ceftriaxone |
| 0.008 | 9 (12.5) | 0 (0.0) | 6 (8.3) | 0 (0.0) |
| 0.015 | 5 (19.4) | 1 (1.4) | 7 (18.1) | 0 (0.0) |
| 0.03 | 3 (23.6) | 8 (12.5) | 0 (18.1) | 8 (11.1) |
| 0.06 | 6 (31.9) | 3 (16.7) | 6 (26.4) | 4 (16.7) |
| 0.12 | 37 (83.3) | 5 (23.6) | 26 (62.4) | 2 (19.4) |
| 0.25 | 8 (94.4) | 3 (27.8) | 19 (88.9) | 3 (23.6) |
| 0.5 | 4 (100.0) | 7 (37.5) | 2 (91.7) | 1 (25.0) |
| 1 | - | 28 (76.5) | 2 (94.4) | 17 (48.6) |
| 2 | - | 12 (93.1) | 0 (94.4) | 28 (87.5) |
| 4 | - | 1 (94.4) | 0 (94.4) | 1 (88.9) |
| 8 | - | 4 (100.0) | 2 (97.2) | 2 (91.7) |
| 16 | - | - | 0 (97.2) | 3 (95.8) |
| 32 | - | - | 0 (97.2) | 0 (95.8) |
| 64 | - | - | 1 (98.6) | 0 (95.8) |
| >64 | - | - | 1 (100.0) | 3 (100.0) |

**Table 2.** MBC/MIC ratios for 50 *S. pneumoniae* strains tested against ceftaroline and ceftriaxone.

| | No. of isolates (cumulative %) at MBC/MIC ratio of: 1 | 2 | 4 | 8 | 16 | ≥32 |
|---|---|---|---|---|---|---|
| Ceftaroline | 47 (65.3) | 18 (90.3) | 3 (94.4) | 0 (94.4) | 0 (94.4) | 4 (100.0) |
| Ceftriaxone | 39 (54.2) | 26 (90.3) | 3 (94.4) | 0 (94.4) | 0 (94.4) | 4 (100.0) |

**Figure 1.** Results of the kill curve experiments testing ceftaroline (Figure 1a) and ceftriaxone (Figure 1b) against a penicillin-resistant *S. pneumoniae* strain.

Figure 1a.

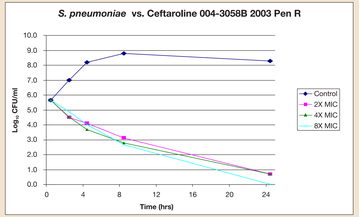


Figure 1b.

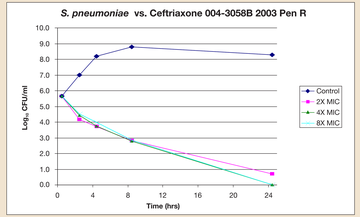


**Table 3.** Kill curve kinetic studies for 12 selected organisms when tested at 4X MIC of ceftaroline and ceftriaxone and monitoring at 2, 4, 8 and 24 hours.

| Organism/antimicrobial | CFU/ml at time indicated (h) 0 | 2 | 4 | 8 | 24 | MIC (µg/ml) |
|---|---|---|---|---|---|---|
| 004-22B | | | | | | |
| Ceftaroline | 4.1E5 | 2.1E4 | 1.5E4 | 2.6E3 | 1.0E0 | 0.008 |
| Ceftriaxone | 4.1E5 | 2.7E4 | 1.1E4 | 1.7E3 | 1.0E0 | 0.03 |
| 064-119B[a] | | | | | | |
| Ceftaroline | 5.1E5 | 3.3E4 | 1.2E4 | 1.1E3 | 2.0E1 | 0.015 |
| Ceftriaxone | 5.1E5 | 3.1E4 | 1.1E4 | 1.2E4 | 5.7E5 | 0.06 |
| 029-2153B | | | | | | |
| Ceftaroline | 2.4E5 | 6.0E4 | 2.2E4 | 9.8E3 | 2.0E2 | 0.5 |
| Ceftriaxone | 2.4E5 | 4.4E4 | 3.5E4 | 8.0E3 | 7.5E1 | 8 |
| 019-2338B | | | | | | |
| Ceftaroline | 3.2E5 | 1.3E4 | 4.5E3 | 9.0E2 | 5.0E0 | 0.12 |
| Ceftriaxone | 3.2E5 | 1.1E4 | 3.7E3 | 6.6E2 | 1.0E0 | 1 |
| 075-2446B | | | | | | |
| Ceftaroline | 4.2E5 | 4.9E4 | 1.6E4 | 3.2E3 | 1.5E1 | 0.25 |
| Ceftriaxone | 4.2E5 | 4.2E4 | 1.3E4 | 2.1E3 | 1.0E1 | 8 |
| 004-3058B | | | | | | |
| Ceftaroline | 4.4E5 | 3.2E4 | 4.6E3 | 5.8E2 | 5.0E0 | 0.25 |
| Ceftriaxone | 4.4E5 | 2.7E4 | 5.3E3 | 5.9E2 | 1.0E0 | 2 |
| 069-3171B | | | | | | |
| Ceftaroline | 4.7E5 | 5.1E4 | 1.4E4 | 2.1E3 | 1.5E2 | 0.25 |
| Ceftriaxone | 4.7E5 | 5.0E4 | 2.0E4 | 3.0E3 | 1.2E2 | 2 |
| 062-3223B | | | | | | |
| Ceftaroline | 4.5E5 | 5.1E4 | 9.9E3 | 2.0E3 | 1.0E0 | 0.12 |
| Ceftriaxone | 4.5E5 | 2.5E4 | 8.6E3 | 1.6E3 | 1.0E0 | 1 |
| 082-3490B | | | | | | |
| Ceftaroline | 5.8E5 | 3.9E4 | 4.5E3 | 7.0E2 | 1.0E1 | 0.12 |
| Ceftriaxone | 5.8E5 | 2.6E4 | 2.1E3 | 4.8E2 | 0.5E1 | 1 |
| 063-3499B | | | | | | |
| Ceftaroline | 1.5E6 | 5.3E5 | 4.8E5 | 7.9E4 | 2.3E2 | 0.12 |
| Ceftriaxone | 1.5E6 | 1.0E6 | 5.0E5 | 1.2E5 | 1.5E2 | 1 |
| 015-3750B | | | | | | |
| Ceftaroline | 6.3E5 | 7.6E4 | 1.1E4 | 1.0E3 | 1.0E0 | 0.12 |
| Ceftriaxone | 6.3E5 | 1.0E5 | 9.0E3 | 1.6E3 | 1.0E0 | 1 |
| 021-3807B | | | | | | |
| Ceftaroline | 5.7E5 | 1.9E4 | 9.7E3 | 1.7E3 | 1.0E0 | 0.12 |
| Ceftriaxone | 5.7E5 | 2.0E4 | 7.9E3 | 1.7E3 | 1.0E0 | 1 |

a. A penicillin-susceptible strain that showed re-growth at 2X ceftaroline MIC and 2X and 4X ceftriaxone MIC. Both cephalosporins were bactericidal (>3 $log_{10}$ reduction in CFU) at 8X MIC (data not shown).

## CONCLUSIONS

- Ceftaroline was eight- to 16-fold more potent than ceftriaxone and highly bactericidal against contemporary *S. pneumoniae* strains.
- High MBC/MIC ratios (≥32) for ceftaroline or ceftriaxone were observed only in four strains (5.6% of the collection), three penicillin-resistant and one penicillin-intermediate.
- Ceftaroline was highly bactericidal against *S. pneumoniae* strains and resistance to penicillin did not adversely influence ceftaroline bactericidal activity.

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