Comparison of Cefepime and Ceftriaxone Bactericidal Activity Against *Streptococcus pneumoniae*

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ABSTRACT

Background: Cefepime (CPM) has increased activity against Enterobacteriaceae compared to third-generation cephalosporins and retains excellent in vitro activity against Gram-positive pathogens, including *S. pneumoniae* (SPN). We compared the bactericidal action of CPM and ceftriaxone (CRO) tested against SPN.

Methods: CPM and CRO MIC and MBC values were determined by CLSI reference broth microdilution methods on 50 recent SPN strains (30 PEN-resistant [R], 10 PEN- intermediate [I] and 10 PEN- susceptible [S]). The lowest concentration that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint. Kill-curve experiments were performed on 14 strains (10 PEN-R, 2 PEN-I and 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 at 24 hours of incubation.

Results: CPM and CRO showed similar in vitro activity with MIC_{50} and MIC_{90} at 1 and 2 µg/ml, respectively, for both drugs. CPM and CRO demonstrated bactericidal activity at ≤2-fold the MIC for 92% and 88% of tested organisms, respectively. CPM kill-curve results are summarized in the table.

	Reduction in $\log_{10}$ CFU/ml at 4X CPM MIC (time in hours)			
Resistance pattern (no.)	2	4	8	24
PEN-S #1	1.3	1.5	2.4	5.6[a]
#2	1.6	1.9	2.6	(0.5)[b]
PEN-I #1	1.3	2.1	3.4	5.5[c]
#2	0.5	0.6	1.0	1.9
PEN-R (10)[c]	1.1	1.6	2.4	4.8

a: No detectable growth; b: Re-growth at 24 hour; c: Average of 10 strains.

CPM showed cidal activity at 8X MIC against 13 (93%) strains. A slower killing pattern was observed in one PEN-I strain with a reduction of 2.1 and 2.0 $\log_{10}$ CFU/ml at 8X CPM and CRO MIC, respectively. One PEN-S strain showed re-growth at 2X and 4X CPM and CRO MIC, while a PEN-R strain showed re-growth at 2X CPM at the 24-hour reading. Generally, bactericidal activities of CPM and CRO were not concentration-dependent except for the 2 strains with re-growth at 2X or 4X MIC on the 24-hour reading.

Conclusions: CPM and CRO were highly and equally bactericidal against SPN strains, and R to PEN did not adversely influence the bactericidal activity of these commonly used cephalosporins.

INTRODUCTION

Streptococcus pneumoniae demands constant vigilance from both clinicians and researchers due to its pathogenicity across all age-groups, ability for colonization and genetic plasticity resulting in dynamic epidemiology and susceptibility trends. The process has been further modulated by interventions such as addition of the heptavalent pneumococcal conjugate vaccine (PCV7) to childhood vaccination schedule and local antimicrobial prescribing practices.

Previous studies have shown that cefepime and ceftriaxone retain excellent antimicrobial activity against global multidrug-resistant (MDR) pneumococcal isolates, with cefepime having significantly lower resistance rates ($P < 0.05$) compared with ceftriaxone (1.3 -1.9% versus 3.0 - 4.4%). Similarly, cefepime and ceftriaxone had extremely low rates of resistance (1.0%) when tested against 157 levofloxacin-non-susceptible (MIC, ≥4 µg/ml) pneumococcal isolates from respiratory sources. The present study further evaluated the bactericidal activity of cefepime and ceftriaxone by testing a contemporary collection of pneumococcal strains.

MATERIALS AND METHODS

Bacterial Isolates: The SENTRY Antimicrobial Surveillance Program monitors the predominant pathogens and their antimicrobial resistance patterns through a broad network of sentinel hospitals in four major geographic regions: Asia/Pacific, Europe, Latin America and the United States/Canada. Fifty recent (2003-2005) pneumococcal strains were selected from the collection obtained through the SENTRY Program. The strains were collected from 37 of the more than 80 participating medical centers. The pneumococci were predominantly isolated from sputum (16; 32.0%), invasive pulmonary infections (14; 28.0%), middle ear fluid (9; 18.0%), upper respiratory tract infections (6; 12.0%) and blood (3; 6.0%). These strains were selected to include a representative sample of *S. pneumoniae* strains stratified by the penicillin susceptibility category: 30 penicillin-resistant (MIC, ≥2 µg/ml); 10 penicillin-intermediate (MIC, 0.12-1 µg/ml) and 10 penicillin-susceptible (MIC, ≤0.06 µg/ml).

Laboratory Testing: To assess the comparative bactericidal activity of cefepime and ceftriaxone, a two-part analysis of the pneumococcal strains was performed. First, minimum inhibitory concentration (MIC) values of cefepime and ceftriaxone for the entire collection of 50 strains were determined by reference broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines and interpretive criteria for non-meningitis pneumococcal strains. Further, minimal bactericidal concentration (MBC) values were determined for cefepime and ceftriaxone by plating the entire volume of broth (100 µl) from the MIC well and from the $\log_2$ dilutions above the MIC for each strain onto appropriate growth media. The lowest concentration of antimicrobial agent that killed ≥99.9% of the initial test inoculum was defined as the MBC endpoint. Secondly, 14 of the 50 pneumococcal strains (10 penicillin-resistant and two each of penicillin-intermediate and penicillin-susceptible strains) were randomly selected for time-kill curve experiments. Time-kill bactericidal activity was performed according to Moody and Knapp [2004] and M26-A methods [NCCLS, 1999]. Two samples of each strain were tested resulting in a total of 28 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} hours.

RESULTS

- Cefepime and ceftriaxone were highly active against penicillin-susceptible (MIC_{90}, 0.06 µg/ml) and penicillin-intermediate (MIC_{90}, 0.5 µg/ml) *S. pneumoniae* strains (100.0% susceptibility).
- Among the penicillin-resistant group, 10 strains (33.3%) showed intermediate MIC values (2 µg/ml) for cefepime and eight strains (26.7%) were intermediate (2 µg/ml) for ceftriaxone. Furthermore, one strain (3.3%) showed a cefepime-resistant MIC value (4 µg/ml), while two strains (6.7%) were resistant to ceftriaxone (Table 1).
- Cefepime MBC values were also nearly identical to those for ceftriaxone (Table 1).
- For the vast majority of strains (48; 96.0%), the MBC/MIC ratios of cefepime and ceftriaxone were ≤8 (MBC/MIC ratios of 1 to 4 for cefepime and 1 to 8 for ceftriaxone; Table 2). Only two strains (4.0%) displayed MBC/MIC ratios ≥32 (indicative of tolerance) for both cefepime and ceftriaxone (one penicillin-resistant and one penicillin-intermediate strain; data not shown).

Table 1. Frequency of occurrence of MIC and MBC values for cefepime and ceftriaxone when tested against 50 *S. pneumoniae* strains.

	No. of isolates (cumulative percentage inhibited) at:			
	MIC		MBC	
Antimicrobial concentration (µg/ml)	Cefepime	Ceftriaxone	Cefepime	Ceftriaxone
0.015	0 (0)	1 (2)	0 (0)	0 (0)
0.03	7 (14)	7 (16)	6 (12)	7 (14)
0.06	3 (20)	2 (20)	1 (14)	3 (20)
0.12	1 (22)	4 (28)	4 (22)	1 (22)
0.25	4 (30)	3 (34)	1 (24)	3 (28)
0.5	6 (42)	3 (40)	3 (30)	1 (30)
1	17 (76)	20 (80)	16 (62)	11 (52)
2	11 (98)	8 (96)	15 (92)	20 (92)
4	1 (100)	0 (96)	2 (96)	0 (92)
8	-	2 (100)	0 (96)	2 (96)
16	-	-	1 (98)	1 (98)
32	-	-	0 (98)	0 (98)
64	-	-	0 (98)	0 (98)
>64	-	-	1 (100)	1 (100)

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

	No. of isolates (cumulative %) at MBC/MIC ratio of:					
	1	2	4	8	16	≥32
Cefepime	32 (64.0)	14 (92.0)	2 (96.0)	0 (96.0)	0 (96.0)	2[a] (100.0)
Ceftriaxone	30 (60.0)	14 (88.0)	3 (94.0)	1 (96.0)	0 (96.0)	2[a] (100.0)

a. One strain (penicillin-intermediate) was evaluated by time kill curve and both cephalosporins showed reduced killing effect (<3 $\log_{10}$ CFU reduction in the initial inoculum; Table 3). The second strain showed intermediate MIC (2 µg/ml) and very high MBC (>64 µg/ml) results for both cephalosporins and was not selected for time kill curve.

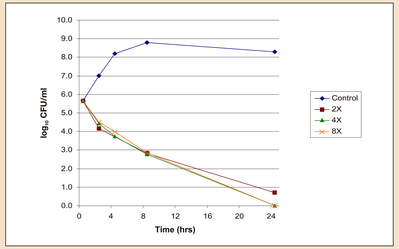
- When tested at 8X MIC, cefepime and ceftriaxone showed bactericidal activity on 13 of 14 strains (92.9%) evaluated by time-kill curve with ≥3 $\log_{10}$ CFU/ml decrease of the initial inoculum (Figure 1).
- Both cephalosporins showed limited bactericidal activity at 2X, 4X and 8X MIC against one penicillin-intermediate strain; however, a reduction of approximately 2 $\log_{10}$ CFU/ml of the initial inoculum was observed (Table 3). This strain exhibited elevated MBC/MIC ratios (≥32) for both cephalosporins.
- One strain (penicillin-susceptible) showed regrowth after 8 hours of exposure at 2X and 4X MIC for both agents, but low MBC/MIC ratios (≤2; Table 3).
- Another strain (penicillin-resistant) demonstrated regrowth after 8 hours at 2X MIC of cefepime. This strain had MIC values of 2 and 8 µg/ml for cefepime and ceftriaxone respectively, and MBC/MIC ratios of 2 for cefepime and 1 for ceftriaxone.

Table 3. Kill curve kinetic studies for 14 selected organisms when tested at 4X MIC of cefepime and monitoring at 2, 4, 8 and 24 hours.

Organism/	CFU/ml at time indicated (h)					MIC
antimicrobial	0	2	4	8	24	(µg/ml)
PEN-S #1	4.1E5	2.1E4	1.2E4	1.7E3	1.0E0	0.03
PEN-S #2[a]	5.1E5	1.4E4	6.3E3	1.3E3	1.5E6	0.06
PEN-I #1	3.3E5	1.6E4	2.4E3	1.2E2	9.0E0	1
PEN-I #2[b]	2.9E5	9.5E4	7.6E4	3.0E4	4.2E3	0.25
PEN-R #1	2.4E5	4.3E4	2.3E4	9.6E3	1.5E2	2
PEN-R #2	3.2E5	9.2E3	3.9E3	6.0E2	1.0E0	1
PEN-R #3	4.2E5	4.1E4	1.0E3	1.9E3	1.0E1	4
PEN-R #4	4.4E5	2.7E4	8.6E3	6.0E2	1.0E0	2
PEN-R #5	4.7E5	5.0E4	1.5E4	3.7E3	1.1E2	1
PEN-R #6	4.5E5	4.3E4	8.8E3	1.6E3	1.0E1	1
PEN-R #7	5.8E5	2.6E4	1.1E3	2.9E2	0.5E1	1
PEN-R #8	1.5R6	8.6E5	4.8E5	1.0E5	1.9E2	2
PEN-R #9	6.3E5	6.7E4	1.1E4	1.2E3	1.0E0	1
PEN-R #10	5.7E5	1.7E4	9.5E4	1.7E3	1.0E0	1

a. Both cephalosporins were bactericidal (≥3 $\log_{10}$ reduction in CFU) at 8X MIC (data not shown).
b. Limited bactericidal activity with both cephalosporins with approximately 2 $\log_{10}$ CFU/ml reduction on the initial inoculum after 24 hours exposure. A penicillin-intermediate strain with MBC/MIC ≥32 for both cephalosporins.

Figure 1. Reduction in ≥3 $\log_{10}$ CFU/ml when a penicillin- susceptible *S. pneumoniae* strain was tested against cefepime by time kill curve.



CONCLUSIONS

- The antimicrobial potency of cefepime was comparable to that of ceftriaxone even when tested against penicillin-resistant pneumococci.
- Cefepime demonstrated good bactericidal activity against a contemporary collection of pneumococcal isolates with varying degrees of penicillin susceptibility, which was comparable to the widely used agent ceftriaxone.
- Cefepime and ceftriaxone represent effective therapeutic alternatives in the treatment of hospitalized patients with invasive pneumococcal infections caused by strains with MDR phenotypes.

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