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Cefepime Alone or in Combination with Clavulanate Potassium, Gentamicin, and Tobramycin Against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Background. Cefepime, a fourth generation cephalosporin with enhanced activity against both gram-positive and gram-negative organisms, has been shown to demonstrate synergistic bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) when combined with antimicrobial agents, such as linezolid, aminoglycoside, and quinupristin/dalfopristin. Preliminary data from our research suggested that the addition of sulbactam, a well-known β-lactamase inhibitor, may increase the activity of cefepime against MRSA. To further evaluate whether other β-lactamase inhibitors may enhance the antimicrobial activity of cefepime, we evaluated clavulanate potassium alone and in combination with cefepime against clinical isolates of MRSA. We also evaluated cefepime alone and in combination with gentamicin or tobramycin against clinical isolates of MRSA.

Methods. Fifty MRSA clinical isolates were analyzed by microbroth dilution for susceptibility to cefepime (CPM), gentamicin (GEN), tobramycin (TOB), clavulanate potassium (CP), as well as cefepime combined with clavulanate potassium 1:1 (CPM-CP), cefepime combined with gentamicin 1:1 (CPM-GEN), and cefepime combined with tobramycin 1:1 (CPM-TOB). Minimum inhibitory concentrations (MIC) were performed according to the Clinical and Laboratory Standard Institute guidelines. Ten of the fifty clinical isolates were selected and evaluated by time-killing curve analysis of CPM, GEN, TOB, CP, CPM-GEN, CPM-TOB, and CPM-CP at 0.5, 1, and 2x the MIC using a starting inoculum of 1 x 10^6 CFU/mL. Synergy (SYN), additivity (ADD), antagonism (ANT), and indifference (IND) was defined as follows: >2.5 log kill, <2.5 but >1 log kill, > 1 log growth and + or - 1 log kill.

Results. MRSA susceptibility to CPM, CP, GEN, TOB, CPM-CP, CPM-GEN, and CPM-TOB are reported as follow [MIC_{50} (range) in mg/L]: 32 (1-64), 64 (32-64), 0.5 (0.13-32), 1 (0.25-32), 32 (4-64), 0.5 (0.25-32), 2 (0.5-64). MBC_{50} (range) in mg/L for CPM, CP, GEN, TOB, CPM-CP, CPM-GEN, and CPM-TOB are 32 (1-64), 64 (32-64), 1 (0.5-32), 2 (0.5-32), 64 (4-64), 1 (0.25-64), 3 (0.5-64). 12% of the isolates demonstrated a decrease in the MIC when CP was added. However, all of the clinical isolates demonstrated a decrease in the MIC when GEN was added (100%) while 54% showed a decrease when TOB was added. Time-killing curve analysis of the clinical isolates showed no difference in log kill for 8 of the organisms tested with CPM and CPM-CP. One organism demonstrated synergy and one showed additivity at 24h when tested with CPM and CPM-CP. Time-killing curve analysis of the same isolates also showed synergy at 24h for 2 organisms when tested with CPM-GEN or CPM-TOB. However, early synergy was noted at 4 or 8 hours in 6 organisms.

Conclusions. A majority of the clinical isolates reported an elevated MIC to CPM. As opposed to sulbactam previously reported, the addition of CP did not appear to enhance the susceptibility of those organisms. In addition, time-killing curve analysis primarily showed indifference with the CPM-CP combination, suggesting that CP may not have as beneficial effect as sulbactam. However, susceptibility is enhanced in all and more than half of the clinical isolates with the addition of GEN and TOB, respectively. The combinations, CPM-GEN and CPM-TOB might warrant further investigation.

BACKGROUND

- Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have led to a significant increase in the use of vancomycin with the consequential development of vancomycin resistance and less treatment options.
- Cefepime is a fourth generation cephalosporin (β-lactam) with enhanced activity against both gram-positive and gram-negative organisms.
- Studies have shown that cefepime demonstrates synergistic bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) when combined with another antimicrobial agent, such as gentamicin, linezolid, or quinupristin/dalfopristin.
- Preliminary data from our research suggested that the addition of sulbactam, a β-lactamase inhibitor, may increase the activity of cefepime against MRSA
- Data from other studies suggest that the addition of clavulanate potassium, a β-lactamase inhibitor, improves the synergistic activity of cefepime against other organisms like *Klebsiella pneumoniae*

OBJECTIVES

- Determine minimum inhibitory concentrations of cefepime alone or in combination with clavulanate potassium, gentamicin, or tobramycin against MRSA clinical isolates
- Evaluate antimicrobial activity of cefepime alone or in combination with clavulanate potassium, gentamicin, or tobramycin in time kill experiments

METHODS

Bacterial Strains
- 50 MRSA clinical isolates were obtained from Anti-infective Research Laboratories, Detroit, MI.

Antibiotics
- Cefepime (Elan Pharmaceuticals, Inc., San Diego, CA)
- Clavulanate potassium (Sigma-Aldrich Chemical Company, St. Louis, MO)
- Gentamicin (Sigma-Aldrich Chemical Company, St. Louis, MO)
- Tobramycin (Sigma-Aldrich Chemical Company, St. Louis, MO)

Medium
- Mueller-Hinton Broth supplemented with magnesium (12.5 mg/L) and calcium (25 mg/L) were used for all microdilution susceptibility testing and in vitro modeling.
- Triptcase Soy Agar were used for growth and colony counts.

Susceptibility Testing
- Minimum inhibitory concentrations (MIC) were determined using microdilution technique with a starting inoculum of 1 x 10^6 CFU/mL according to the Clinical and Laboratory Standard Institute guidelines, and incubated for 24 hours at 35ºC.

METHODS (CONT.)

Time-Kill Experiments
- 10 clinical isolates were selected for time-kill analysis
- Experiments were performed with a starting inoculum of 1 x 10^6 CFU/mL
- All antimicrobials were tested alone and in combination at 0.5, 1, and 2x the MIC of each isolate
- Synergy (SYN), additivity (ADD), antagonism (ANT), and indifference (IND) was defined as follows: >2.5 log kill, <2.5 but >1 log kill, > 1 log growth and + or - 1 log kill
- Antibiotic carryover was minimized by filtration and/or serial dilution for those concentration that were tested close to the MIC.

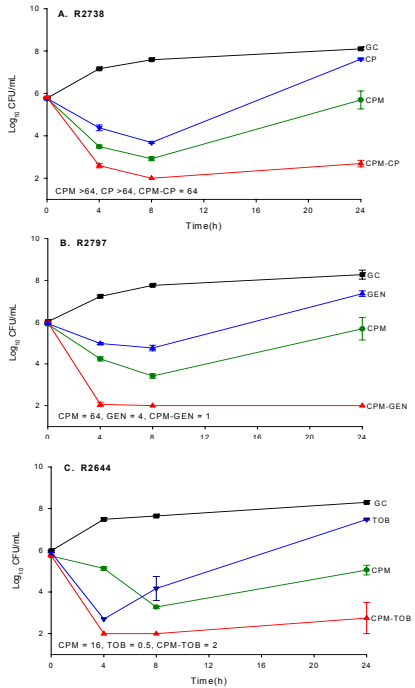
RESULTS

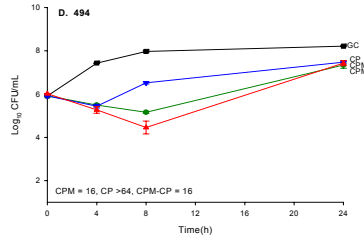
	MIC (mg/L)			MBC (mg/L)		
Agent	**50%**	**90%**	**Range**	**50%**	**90%**	**Range**
CPM	32	64	1-64	32	64	1-64
CP	64	64	32-64	64	64	32-64
GEN	0.5	4	0.13-32	1	8	0.5-32
TOB	1	4	0.25-32	2	8	0.5-32
CPM-CP	32	64	4-64	64	64	4-64
CPM-GEN	0.5	2	0.25-32	1	2	0.25-64
CPM-TOB	2	64	0.5-64	3	64	0.5-64

0.5X MIC		% SYN (mean ▲CFU/mL ± std)	% ADD (mean ▲CFU/mL ± std)	% IND (mean ▲CFU/mL ± std)	% ANT (mean ▲CFU/mL ± std)
CPM-CP	4h	----	----	100% (-0.47 ± 0.21)	----
	8h	----	----	100% (-0.32 ± 0.32)	----
	24h	10% (-3.0)	10% (-1.73)	80% (-0.35 ± 0.42)	----
CPM-GEN	4h	20% (-3.94 ± 1.5)	40% (-1.88 ± 0.28)	30% (-0.47 ± 0.29)	10% (2.57)
	8h	10% (-3.0)	20% (-1.67 ± 0.35)	20% (0.04 ± 0.82)	50% (2.06 ± 1.54)
	24h	10% (-3.68)	----	60% (-0.01 ± 0.4)	30% (3.96 ± 0.9)
CPM-TOB	4h	50% (-3.46 ± 0.96)	30% (-1.81 ± 0.55)	20% (0.28 ± 0.22)	----
	8h	10% (-4.22)	40% (-1.85 ± 0.37)	30% (-0.29 ± 0.59)	20% (1.52 ± 0.15)
	24h	30% (-5.54 ± 1.62)	20% (-1.85 ± 0.64)	40% (-0.11 ± 0.85)	10% (1.15)

Figure 1. Time kill results of three clinical MRSA isolates at 0.5X MIC showing enhanced antimicrobial activity of cefepime with A) clavulanate potassium (CPM-CP), B) gentamicin (CPM-GEN), C) tobramycin (CPM-TOB). Time kill results showing indifference in antimicrobial activity with CPM-CP at 0.5X MIC (D).

RESULTS





- 12% of clinical MRSA isolates demonstrated decrease in MIC with the CPM-CP combination
- 100% and 54% of clinical MRSA isolates demonstrated decrease in MIC with addition of GEN and TOB to CMP, respectively
- Indifference was noted in majority of the isolates tested with CPM and CPM-CP in time-kill studies
- Early synergy was noted at 4 hours when tested with CPM-GEN and CPM-TOB in time-kill studies

CONCLUSIONS

- Unlike sulbactam, the addition of CP did not appear to enhance the susceptibility of MRSA clinical isolates to cefepime
- Time-kill studies show primarily indifference with the CPM-CP combination
- However, susceptibility was enhanced in all and more than half of the clinical isolates with the addition of GEN and TOB to CPM, respectively
- Further in vitro studies with the CPM-CP, CPM-GEN, CPM-TOB combinations on a larger population of isolates might be warranted

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