F-2130

IN VITRO ACTIVITY OF MC-1: A NEW GRAM-NEGATIVE ANTIMICROBIAL AGENT

D.J. FARRELL[1], D.J. BIEDENBACH[1], J.P. MUELLER[2], J.P. QUINN[2], R.N. JONES[1]
JMI Laboratories, North Liberty, Iowa, USA; [2]Pfizer Global R&D, Groton, CT, USA

ICAAC 2010
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, 319.665.3371
david-farrell@jmilabs.com

AMENDED ABSTRACT

Background: MC-1 is a novel siderophore monocarbam antimicrobial agent targeted at Gram-negative (GN) pathogens including multidrug-resistant (MDR) strains. In this preliminary study, the in vitro activities of MC-1 were evaluated against contemporary wild-type and MDR-GN pathogens.

Methods: *K. pneumoniae* (KPN, 100 strains), *E. coli* (EC, 80 strains), *Enterobacter* spp. (ES, 27 strains), *Citrobacter* spp. (CS, 26 strains), *Serratia marcescens* (SM, 30 strains), *Proteus* spp. (PRS, 26 strains), *P. aeruginosa* (PSA, 101 strains), and *Stenotrophomonas maltophilia* (SMAL, 26 strains) were collected in 2009 from geographically diverse United States (USA) medical centers. Isolates were selected to mimic current antimicrobial resistance prevalence using 4th generation cephalosporin and carbapenem MIC distributions. Strains resistant to cefepime and meropenem were included in both pathogen groups. MC-1 and comparators were tested for potency and susceptibility by the CLSI broth microdilution methodology (M07-A8; M100-S20).

Results: Overall MC-1 potency was high against 416 selected Gram-negative strains with 97.1% of strains inhibited at a MIC of ≤4 μg/ml. MC-1 showed MIC_{90} values of only 2, 2, 4, 2, 0.5, 0.12, 0.5 and 4 μg/ml against KPN, EC, ES, CS, SM, PRS, PSA and SMAL, respectively. MC-1 inhibited all 12 *E. coli* harboring CTX-M genes at a MIC ≤8 μg/ml. MC-1 potency was equivalent or higher than the comparator agents tested for most strains.

Conclusions: MC-1 demonstrated potent in vitro activity against contemporary (2009) USA strains of Gram-negative pathogens including cefepime- and meropenem- resistant isolates. This promising activity compared to contemporary broad-spectrum β-lactams should be further evaluated against other species, including those with known resistance mechanisms.

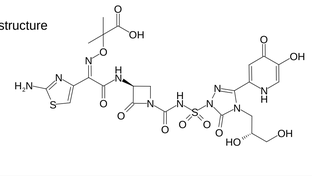
INTRODUCTION

Due to recent medical advances, such as transplantation and immunosuppressive therapy, prolonged stays for patients in hospital and long-term care facilities are becoming more common. Concurrently, increases in healthcare-associated infections (HAIs) have been observed. Multidrug-resistant (MDR) Gram-negative bacilli frequently cause HAIs and antimicrobial resistance in Gram-negative pathogens is associated with increased morbidity and mortality.

In the last few decades, we have witnessed the rapid spread of extended-spectrum β-lactamase (ESBL)-encoding genes in *Escherichia coli* and *Klebsiella pneumoniae* together with an overall rise in the prevalence of fluoroquinolone resistance. In particular, the dissemination of CTX-M enzymes in Enterobacteriaceae has been reported worldwide in recent years. In the clinical setting where ESBLs are prevalent, carbapenems are the therapeutic option usually chosen. Complicating this, carbapenem therapy has been challenged by the rise of metallo-β-lactamase (MBL; e.g. VIM-, IMP-, NDM-)- and KPC-producing Enterobacteriaceae, further limiting therapeutic options. Unfortunately, there is also a recognized lack of development of novel compounds targeting Gram-negative pathogens responsible for HAIs.

MC-1 (Figure 1) is a novel siderophore monocarbam antimicrobial agent targeted at Gram-negative (GN) pathogens, including MDR strains. The aim of this study was to provide a preliminary evaluation of the activity of MC-1 against a contemporary collection of clinically relevant Gram-negative pathogens collected from geographically diverse medical centers in the United States (USA).

Figure 1.
Chemical structure of MC-1.



MATERIALS AND METHODS

Bacterial strain collection. A total of 416 non-duplicate Gram-negative clinical isolates collected in 2009 from geographically diverse USA medical centers were selected to mimic current antimicrobial resistance prevalence using 4th generation cephalosporin (cefepime) and carbapenem (meropenem) distributions. Sources of recovered organisms included bloodstream (59.2%), respiratory tract (15.6%), skin and skin-structure specimens (8.9%), and other clinical specimen types (16.4%). Twelve CTX-M (six CTX-M-14 and six CTX-M-15) genotypically characterized *E. coli* strains were selected to specifically assess the in vitro activity of MC-1 against strains harbouring these increasingly prevalent genes/enzymes. Species identifications were confirmed by JMI Laboratories using standard algorithms and the automated Vitek 2 system (bioMérieux, Hazelwood, Missouri, USA), when necessary.

Antimicrobial susceptibility testing. Isolates were tested for susceptibility by the reference broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) recommendations. Susceptibility testing was performed by using investigator-prepared 96-well frozen-form panels with cation-adjusted Mueller-Hinton broth. Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S20, 2010) quality control (QC) strains: *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Interpretation of comparator MIC results was in accordance with published CLSI (M100-S20) breakpoint criteria.

RESULTS

- Overall, MC-1 potency was high against 416 selected Gram-negative strains with 97.1% of strains inhibited at a MIC of ≤4 μg/ml (Table 1).
- MC-1 was very active against 100 *K. pneumoniae* strains with a $MIC_{50/90}$ of 0.06/2 μg/ml. Resistance to comparator antimicrobials was 12.0, 4.0, 14.0, and 13.0% for cefepime, meropenem, aztreonam and ciprofloxacin, respectively (Table 2). Four *K. pneumoniae* strains had MC-1 MIC values of ≥8 μg/ml; two of these strains with MIC values of 32 μg/ml (Table 1).
- MC-1 ($MIC_{50/90}$, 0.12/2 μg/mL) inhibited all *E. coli* at ≤16 μg/ml, whereas cefepime, meropenem, aztreonam and ciprofloxacin resistance rates were 15.0, 1.3, 21.3 and 45.0%, respectively (Table 2). MC-1 inhibited all 12 *E. coli* harboring CTX-M genes at a MIC ≤8 μg/ml.
- MC-1 demonstrated good activity against the other Enterobacteriaceae tested with $MIC_{50/90}$ values of 0.5/4, 0.25/2, 0.12/0.5, and ≤0.03/0.12 μg/ml against *Enterobacter* spp., *Citrobacter* spp., *Serratia marcescens*, and *Proteus* spp., respectively (Table 2).
- MIC values for MC-1 against *P. aeruginosa* ranged from ≤0.03 to 64 μg/ml with very potent $MIC_{50/90}$ values of 0.25/0.5 (Tables 1 and 2). MC-1 (MIC_{90}, 0.5 μg/ml) was the most active agent tested against these strains compared to cefepime (MIC_{90}, 16 μg/ml), meropenem (MIC_{90}, 4 μg/ml), aztreonam (MIC_{90}, >16 μg/ml) and ciprofloxacin (MIC_{90}, 4 μg/ml).
- MC-1 was also the most potent antimicrobial agent tested against 26 strains of *Stenotrophomonas maltophilia* (MIC_{90}, 4 μg/ml) compared to cefepime (MIC_{90}, 64 μg/ml), meropenem (MIC_{90}, >8 μg/ml), aztreonam (MIC_{90}, >16 μg/ml) and ciprofloxacin (MIC_{90}, >4 μg/ml).

Table 1. MIC frequency distribution for MC-1 against 416 clinical isolates of Gram-negative bacilli

	Number of strains inhibited at MIC value (μg/ml) of:										
Organism (n)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
K. pneumoniae (100)	47	7	10	13	5	4	7	3	1	1	2	-
E. coli (80)	29	7	10	6	9	9	2	5	2	1	-	-
CTX-M-14 (6)	4	-	-	-	1	-	-	-	1	-	-	-
CTX-M-15 (6)	-	-	-	-	-	3	1	2	-	-	-	-
Enterobacter spp. (27)[a]	2	2	4	5	3	4	3	4	2	-	-	-
Citrobacter spp. (26)[b]	2	2	5	5	3	5	2	1	-	-	1	-
Serratia marcescens (30)	6	7	9	3	2	2	1	-	-	-	-	-
Proteus spp. (26)[c]	19	4	2	1	-	-	-	-	-	-	-	-
P. aeruginosa (101)	9	12	28	28	16	5	2	-	-	-	-	1
Stenotrophomonas maltophilia (26)	-	-	-	1	3	13	6	3	-	-	-	-

a. Includes: *E. aerogenes* (six isolates), and *E. cloacae* (21 isolates).
b. Includes: *C. amalonaticus* (two isolates), *C. braakii* (two isolates), *C. freundii* (15 isolates), *C. koseri* (five isolates), and unspeciated *Citrobacter* (two isolates).
c. Includes: *P. mirabilis* (23 isolates), and *P. vulgaris* (3 isolates).

Table 2. Antimicrobial activity of MC-1 and comparator antimicrobial agents against 416 clinical isolates of Gram-negative bacilli

Organism (n)/ antimicrobial agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %R
K. pneumoniae (100)				
MC-1	0.06	2	≤0.03 – 32	-/-[b]
Cefepime	≤0.06	32	≤0.06 – >64	87.0 / 12.0
Meropenem	≤0.12	≤0.12	≤0.12 - >8	95.0 / 4.0
Aztreonam	≤0.12	>16	≤0.12 - >16	85.0 / 14.0
Ciprofloxacin	≤0.5	4	≤0.5 - >4	84.0 / 13.0
E. coli (80)				
MC-1	0.12	2	≤0.03 – 16	- / -
Cefepime	≤0.06	>64	≤0.06 – >64	81.3 / 15.0
Meropenem	≤0.12	≤0.12	≤0.12 – 4	98.8 / 1.3
Aztreonam	0.25	>16	≤0.12 – >16	73.8 / 21.3
Ciprofloxacin	≤0.5	>4	≤0.5 – >4	55.0 / 45.0
Enterobacter spp. (27)[c]				
MC-1	0.5	4	≤0.03 – 8	- / -
Cefepime	0.12	2	≤0.06 – >64	92.6 / 7.4
Meropenem	≤0.12	0.25	≤0.12 – 4	92.6 / 7.4
Aztreonam	0.5	>16	≤0.12 – >16	63.0 / 29.6
Ciprofloxacin	≤0.5	≤0.5	≤0.5 – >4	92.6 / 7.4
Citrobacter spp. (26)[d]				
MC-1	0.25	2	≤0.03 – 32	- / -
Cefepime	≤0.06	2	≤0.06 – 2	100.0 / 0.0
Meropenem	≤0.12	≤0.12	≤0.12	100.0 / 0.0
Aztreonam	≤0.12	>16	≤0.12 – >16	65.4 / 34.6
Ciprofloxacin	≤0.5	1	≤0.5 – 4	92.3 / 3.8
Serratia marcescens (30)				
MC-1	0.12	0.5	≤0.03 – 2	- / -
Cefepime	0.25	8	≤0.06 – 32	93.3 / 3.3
Meropenem	≤0.12	8	≤0.12 – >8	86.7 / 13.3
Aztreonam	0.25	>16	≤0.12 – >16	63.3 / 23.3
Ciprofloxacin	≤0.5	>4	≤0.5 – >4	76.7 / 20.0
Proteus spp. (26)[e]				
MC-1	≤0.03	0.12	≤0.03 – 0.25	- / -
Cefepime	≤0.06	0.12	≤0.06 – 1	100.0 / 0.0
Meropenem	≤0.12	≤0.12	≤0.12	100.0 / 0.0
Aztreonam	≤0.12	≤0.12	≤0.12 – 1	100.0 / 0.0
Ciprofloxacin	≤0.5	>4	≤0.5 – >4	57.7 / 34.6
P. aeruginosa (101)				
MC-1	0.25	0.5	≤0.03 - 64	-/-
Cefepime	2	16	0.5 - >64	86.1 / 6.9
Meropenem	0.5	4	≤0.12 - >8	90.1 / 6.9
Aztreonam	4	>16	0.25 – >16	74.3 / 12.9
Ciprofloxacin	≤0.5	4	≤0.5 - >4	76.2 / 17.8
S. maltophilia (26)				
MC-1	1	4	0.25 – 4	- / -
Cefepime	32	64	4 – 64	- / -
Meropenem	>8	>8	>8	- / -
Aztreonam	>16	>16	16 – >16	- / -
Ciprofloxacin	4	>4	1 – >4	- / -

a. Criteria as published by CLSI (2010).
b. - reads no breakpoint criteria are available.
c. Includes: *E. aerogenes* (six isolates), and *E. cloacae* (21 isolates).
d. Includes: *C. amalonaticus* (two isolates), *C. braakii* (two isolates), *C. freundii* (15 isolates), *C. koseri* (five isolates), and unspeciated *Citrobacter* (two isolates).
e. Includes: *P. mirabilis* (23 isolates), and *P. vulgaris* (3 isolates).

CONCLUSIONS

- MC-1 demonstrated promising in vitro activity against contemporary Gram-negative pathogens tested in this study, including *E. coli* harboring CTX-M-14 and -15 ESBLs.
- MC-1 showed greater activity compared to the comparator agents when tested against *P. aeruginosa* and *S. maltophilia* isolates.
- In summary, MC-1 exhibited high potency against this contemporary collection of Gram-negative pathogens from the USA. These in vitro data warrant further investigations with MC-1 to determine if these findings indicate a potential therapeutic option.

ACKNOWLEDGEMENT

This research study was supported by Pfizer, Inc.

REFERENCES

1. Castanheira M, Mendes RE, Rhomberg PR, Jones RN (2008). Rapid emergence of blaCTX-M among Enterobacteriaceae in U.S. Medical Centers: Molecular evaluation from the MYSTIC Program (2007). Microb Drug Resist 14: 211-216.
2. Centers for Disease Control and Prevention (2010). Detection of Enterobacteriaceae isolates carrying metallo-β-lactamase - United States, 2010. MMWR Morb Mortal Wkly, 59: 750, 2010.6.
3. Cornaglia G, Rossolini GM. The emerging threat of acquired carbapenemases in Gram-negative bacteria. Clin Microbiol Infect 16: 99-101.
4. Clinical and Laboratory Standards Institute (2009a). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - eighth edition*. Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2010). *M100-S20. Performance standards for antimicrobial susceptibility testing. 20th informational supplement.* Wayne, PA: CLSI.
6. Paterson DL (2008). Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin Infect Dis. 15;47 Suppl 1:S14-20.