

# In Vitro Activity of CEM-101 Against Resistant Strains of *Staphylococcus aureus*

J. DUBOIS<sup>1\*</sup>, P. FERNANDES<sup>2</sup><sup>1</sup> CSSS Coaticook, Sherbrooke, Qué., Canada, <sup>2</sup> Cempra Pharmaceuticals Inc., Chapel Hill, NCICAAC 2009  
Jacques Dubois Ph.D.  
M360 and CSSS Coaticook  
Sherbrooke, Québec, Canada  
819.571.4366 fax 819.843.1391  
jdubois@m360.ca

## Revised Abstract

**Background:** CEM-101 is a promising fluoroketolide that has potent activity against bacterial pathogens resistant to other macrolide agents. The activity against a variety of resistant strains of *Staphylococcus aureus* was investigated.

**Methods:** The *in vitro* activity of CEM-101 was compared with that of telithromycin, azithromycin, erythromycin, levofloxacin, linezolid and doxycycline against a total of 272 resistant *S aureus* by agar dilution procedures (CLSI, M7-A7, M100-S18). The tested strains included *S. aureus* MRSA (Mec A genotype; 176 isolates), macrolide-resistant (*ermA*, B, C genotype or MLSb-resistant; 58) and ciprofloxacin-resistant (*gyrA* and *parC* genotype; 38).

**Results:** Against *S. aureus* MRSA (MecA), CEM-101 (MIC<sub>90</sub> 0.06 mg/L) and telithromycin (MIC<sub>90</sub> 0.06 mg/L) were more active than doxycycline (MIC<sub>90</sub> 1 mg/L), linezolid (MIC<sub>90</sub> 2 mg/L), levofloxacin (MIC<sub>90</sub> 16 mg/L), azithromycin (MIC<sub>90</sub> >32 mg/L) and erythromycin (MIC<sub>90</sub> >32 mg/L). CEM-101 (MIC<sub>90</sub> 0.06 mg/L) was significantly superior to linezolid (MIC<sub>90</sub> 2 mg/L), levofloxacin (MIC<sub>90</sub> 4 mg/L), telithromycin (MIC<sub>90</sub> 4 mg/L), azithromycin (MIC<sub>90</sub> >32 mg/L), and erythromycin (MIC<sub>90</sub> >32 mg/L) against macrolide-resistant *S. aureus* (*ermA*, B, C genotype or MLSb-resistant). Against ciprofloxacin-resistant (*gyrA* and *parC* genotype) *S. aureus*, erythromycin (MIC<sub>90</sub> >32 mg/L), levofloxacin (MIC<sub>90</sub> >32 mg/L), azithromycin (MIC<sub>90</sub> 32 mg/L), linezolid (MIC<sub>90</sub> 2 mg/L), and doxycycline (MIC<sub>90</sub> 1 mg/L) were less active than CEM-101 (MIC<sub>90</sub> 0.06 mg/L) and telithromycin (MIC<sub>90</sub> 0.06 mg/L).

**Conclusions:** These data confirms the potent activity of this new fluoroketolide, CEM-101, against resistant *S. aureus*.

## Introduction

CEM-101 is a novel fluoroketolide antibacterial agent related to 14-membered ring macrolides. CEM-101 appears to exhibit superior ability to bind to the ribosomes dimethylated at 2058 by the action of *erm* methyltransferase.

In susceptibility studies, CEM-101 is appreciably more potent than most macrolides or azalides against many Gram-positive organisms, including resistant *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus* spp. It has potent activity against various atypical respiratory pathogens like *Legionella pneumophila*, *Mycoplasma* spp. and *Chlamydia* spp.

## Objective

We determined the minimum inhibitory concentration (MIC) of CEM-101, telithromycin, azithromycin, erythromycin, levofloxacin, linezolid and doxycycline against a variety of *Staphylococcus aureus* strains isolated from patient sources.

## Materials and Methods

### Strains

- A variety of recent strains (1995-2008) of *Staphylococcus aureus* were isolated, mostly from upper or lower respiratory tract, blood culture or wounds.
- Multiple cultures from the same patient or source were excluded unless a change in organism or antibiogram was noted.
- Organisms were identified by standard methods such as described by Murray et al (1).

Microorganisms	Number of tested strains
<i>Staphylococcus aureus</i>	272
-Methicillin-resistant (mec A genotype)	176
-Macrolide-resistant ( <i>ermA</i> , B, C genotype)	58
-Ciprofloxacin-resistant ( <i>gyrA</i> and <i>parC</i> genotype)	38

### Determination of MICs

- MICs were determined using the CLSI agar dilution method (2, 3), with replicate plating of the organisms onto a series of agar plates of increasing concentrations from 0.004 mg/L to 64 mg/L.
- Mueller-Hinton agar was used as the medium against *S. aureus* strains.
- Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC25922 were included as controls.

### Determinations of genotype *mec A*, *ermA*, B, C, *mefE* and *gyrA* and *parC*

- Genomic DNA was isolated as described by Smith et al (4)
- Multiplex PCR was performed with primers specific for *mec A*, *ermA*, *ermB*, *ermC* and *mefE* as described by Sutcliffe et al (5)
- Multiplex PCR was performed with primers specific for *gyrA* and *parC* as described by Gonzalez et al (6)

## Results

- Susceptibility of *Staphylococcus aureus* to CEM-101 compared with those of other macrolides, levofloxacin, linezolid or doxycycline is shown in Table 1.
- The activity of CEM-101 (MIC<sub>90</sub> 0.06 mg/L) was similar to that of telithromycin (MIC<sub>90</sub> 0.06 mg/L), but it was significantly more potent than other macrolides tested (azithromycin and erythromycin, MIC<sub>90</sub> ≥64 mg/L), a quinolone (levofloxacin, MIC<sub>90</sub> 16 mg/L), linezolid (MIC<sub>90</sub> 2 mg/L) and doxycycline (MIC<sub>90</sub> 1 mg/L) against *S. aureus* methicillin-resistant (Mec A phenotype) strains.
- Against *S. aureus* erythromycin-resistant group (*ermA*, B, C genotype), CEM-101 (MIC<sub>90</sub> 0.06 mg/L) was the most active compound tested. Moreover, the activity of telithromycin (MIC<sub>90</sub> 4 mg/L), azithromycin (MIC<sub>90</sub> ≥64 mg/L), levofloxacin (MIC<sub>90</sub> 4 mg/L), linezolid (MIC<sub>90</sub> 2 mg/L) and doxycycline (MIC<sub>90</sub> 1 mg/L) was significantly lower than the activity of CEM-101.
- Against *S. aureus* ciprofloxacin-resistant (*gyrA* and *parC* genotype) pathogens, CEM-101 (MIC<sub>90</sub> 0.06 mg/L) and telithromycin (MIC<sub>90</sub> 0.06 mg/L) were the most active compounds tested followed by doxycycline (MIC<sub>90</sub> 1 mg/L), linezolid (MIC<sub>90</sub> 2 mg/L), azithromycin (MIC<sub>90</sub> 32 mg/L), erythromycin (MIC<sub>90</sub> ≥64 mg/L) and levofloxacin (MIC<sub>90</sub> ≥64 mg/L).

TABLE 1. Susceptibility of *Staphylococcus aureus*

Organism (no. tested)	Antibiotic	MIC (mg/L)		
		Range	50%	90%
<i>S. aureus</i> Methicillin-R MecA (176)	CEM-101	0.008-32	0.06	0.06
	Telithromycin	0.016-32	0.06	0.06
	Azithromycin	0.12-≥64	2	≥64
	Erythromycin	0.016-≥64	1	≥64
	Levofloxacin	1-16	4	16
	Linezolid	0.5-4	1	2
<i>S. aureus</i> Macrolide-R <i>ermA,B,C</i> (58)	CEM-101	0.008-0.12	0.06	0.06
	Telithromycin	0.12-16	2	4
	Azithromycin	4-≥64	32	≥64
	Erythromycin	8-≥64	≥64	≥64
	Levofloxacin	0.5-4	1	4
	Linezolid	1-4	1	2
<i>S. aureus</i> Ciprofloxacin-R <i>gyrA, parC</i> (38)	CEM-101	0.016-0.25	0.06	0.06
	Telithromycin	0.016-0.25	0.03	0.06
	Azithromycin	0.016-≥64	0.12	32
	Erythromycin	0.06-≥64	1	≥64
	Levofloxacin	8-≥64	≥64	≥64
	Linezolid	1-4	2	2
	Doxycycline	0.5-1	1	1

## Discussion

- CEM-101 showed significant activity (MIC<sub>90</sub> 0.06 mg/L) against categorized *Staphylococcus aureus* strains, including strains that were resistant to β-lactams, macrolides or quinolones.
- CEM-101 was the only antimicrobial agent that inhibited 90% of all resistant strains of *S. aureus* tested at a constant concentration of 0.06 mg/L.
- Activity of CEM-101 against *S. aureus* was significantly superior (p<0.05) to levofloxacin, the quinolone tested.
- Activity of CEM-101 was also significantly superior to the macrolides tested, azithromycin and erythromycin, and was much more potent than linezolid and doxycycline.
- Against erythromycin-resistant (*ermA*, B, C genotype) *S. aureus* strains, CEM-101 (MIC<sub>90</sub> 0.06 mg/L) was still the most active agent tested and was significantly more active than all other macrolides tested (telithromycin, azithromycin and erythromycin (MIC<sub>90</sub> >4 mg/L).

## Conclusion

- CEM-101 shows a broad spectrum of activity against the most commonly isolated resistant strains of *S.aureus* isolated from wound infections.
- With favorable pharmacokinetics in humans, CEM-101 should be a valuable oral compound for the treatment of *Staphylococcus aureus* infections caused by *S. aureus* that are resistant to standard oral macrolides, quinolones, or β-lactams.
- Clinical studies should undertaken to evaluate the *in vivo* effectiveness of this new antimicrobial agent.

## References

- Murray et al., Manual of Clinical Microbiology, 9rd ed., 2007, A.S.M. Chap. 28; 390-411.
- Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement; M100-S18, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, January 2008)
- Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard 17<sup>th</sup> edition, M7-A7, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, 2006)
- Smith et al, Antimicrob. Ag. Chemo.; 37, 1938-1944, 1993
- Sutcliffe et al, Antimicrob. Ag. Chemo.; 40, 2562-2566, 1996
- Gonzalez et al, Antimicrob. Ag. Chemo.; 42, 2792-2798, 1998