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# Antimicrobial Characterization of CEM-101: Activity Against Enterococci, Uncommon Gram-positive Pathogens, *N. gonorrhoeae* and Anaerobes

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MIC (µg/ml)

Range

90%

50%

Organism (no. tested)/

antimicrobial agent

Bacillus spp.(10)<sup>a</sup>

#### AMENDED ABSTRACT

**Background:** CEM-101, a new macrolide-ketolide, has potency advantages over other MLS<sub>B</sub>-ketolides against pathogens causing respiratory tract and cutaneous infections. However, expanded activity vs. other species may be clinically helpful, and those species were tested.

**Methods:** Four major organism groups (257 strains) were processed including: *enterococci* (99 [Table]; 39 *E. faecalis*, 40 *E. faecium*, 20 VRE), *N. gonorrhoeae* (34; 44% ciprofloxacin [CIP]-resistant [R]), anaerobes (71; 7 genus or species groups) and unusual Gram-positive species (53, 5 organism groups). All susceptibility (S) testing used CLSI broth microdilution or agar dilution methods and interpretations. 5-11 comparison agents were tested including telithromycin (TEL) and azithromycin (AZ).

**Results:** CEM-101 potency against enterococci showed a bimodal distribution (0.03 and 2 μg/ml), and that activity was 2- and ≥32-fold superior to TEL and AZ, respectively. Against *E. faecalis* CEM-101 activity (MIC<sub>90</sub>, 2 μg/ml) was like amoxicillin/clavulanate (MIC<sub>90</sub>, 1 μg/ml) and linezolid (MIC<sub>90</sub>, 2 μg/ml). CEM-101 MIC<sub>90</sub> for *Bacillus* spp., *Listeria* spp. and *Micrococcus* spp. was 0.03 μg/ml, but 0.5 μg/ml for *Corynebacterium* spp. Gonococci had all CEM-101 MIC results at ≤0.25 μg/ml, 4-fold more potent than AZ. Anaerobe CEM-101 MIC<sub>90</sub> results varied from >32 (*B. fragilis, C. difficile*) to ≤0.25 μg/ml (Gram-positive [GP] spp).

	CEM	-101 MIC (	μg/ml)	TE	/ml)	
Organism/resistance	50%	90%	Range	50%	90%	Range
E. faecalis						
Vancomycin-S (29)	0.03	2	0.015-2	0.06	4	0.03-8
Vancomycin-R (10)	0.25	2	0.015-2	0.5	4	0.03-4
E. faecium						
Vancomycin-S (30)	0.25	2	0.03-2	0.5	4	0.03-8
Vancomycin-R (10)	2	2	0.25-2	4	4	2-4
Other enterococci (20)	0.03	1	0.015-2	0.06	2	0.015-4

**Conclusions:** CEM-101 exhibited varied activity against tested species, however clinical utility could be expected against most enterococci, *N. gonorrhoeae*, GP anaerobes and most uncommonly isolated GP species. Wider CEM-101 clinical applications should be considered.

#### INTRODUCTION

CEM-101 is a novel macrolide-ketolide agent with potent activity against pathogens causing community-acquired respiratory tract infections (CA-RTI; *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) and uncomplicated skin and skin structure infections (uSSSI; *Staphylococcus aureus*, ß-haemolytic streptococci). Although antimicrobial agents in this class have been targeted for use in these cited indications, the CEM-101 spectrum may be compatible for treatment of other infection types and species. Emerging resistance mechanisms and greater occurrence of existing resistance rates to the MLS<sub>B</sub>-ketolides, have further limited treatment options.

In this in vitro study, CEM-101 was tested by reference dilution methods against contemporary isolates of enterococci, uncommonly cultured Grampositive species, *Neisseria gonorrhoeae* and prevalent strict anaerobic organisms. These results could guide the clinical program to investigate CEM-101 for expanded indications, especially for sexually transmitted disease (STD; gonorrhea) where resistances to ß-lactams, tetracyclines and fluoroquinolones have severely limited effective therapies.

## MATERIALS AND METHODS

Susceptibility testing methods: Clinical and Laboratory Standards Institute (CLSI) methods were used for all testing as follows:

(53 strains), the M7-A7 (2006) broth microdilution method in Mueller-Hinton broth

For Enterococcus spp. and uncommonly isolated Gram-positive species

- For N. gonorrhoeae, the M7-A7 (2006) agar dilution method on GC agar with 1% defined supplement
- For anaerobes, the M11-A7 (2007) agar dilution method on Brucella laked blood agar

CEM-101 and 16 selected antimicrobial agents from 10 classes were tested. Results were validated by testing the following CLSI-recommended quality control (QC) strains: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *N. gonorrhoeae* ATCC 49226, *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741 and *C. difficile* ATCC 700057. All QC values were within published ranges.

Organisms tested: All organisms tested were collected from patients in USA and European medical centers from 2005 to present. Sources of recovered isolates included bloodstream, uSSSI, CA-RTI, genitourinary and gastrointestinal tract. Unusual/rare organism species and phenotypes required use of strains isolated prior to 2005 or from other geographic areas. The following organisms were tested (see Tables 1-4):

- *N. gonorrhoeae* (34; ≥5 ß-lactamase-positive and approximately 40% resistant to penicillin, tetracycline and fluoroquinolones [ciprofloxacin])
- Enterococci (39 E. faecalis, 10 vancomycin-resistant; 40 E. faecium, 10 vancomycin-resistant; 20 other Enterococcus spp., five species)
- Bacillus spp. (10, three species)
- Corynebacterium spp. (10, four species)
- L. monocytogenes (10)
- Micrococcus spp. (10, two species)
- Other rare Gram-positive isolates (13, nine species)
- Anaerobic organisms (71; seven genus or species groups)
- A total of 257 strains were tested, each identified by at least two laboratories including a reference GLP facility (JMI Laboratories, North Liberty, Iowa).

#### RESULTS

- CEM-101 was tested against 99 recent enterococcal isolates (Table 1) and greatest activity (MIC<sub>50</sub>) was detected against *E. faecalis* (0.03-0.25 μg/ml), vancomycin-susceptible *E. faecium* and other *Enterococcus* spp. (0.03 μg/ml). Vancomycin-resistant strains were generally less inhibited by CEM-101 and a bimodal distribution of MIC values (Table 4) was observed correlating to the erythromycin susceptibility patterns.
- Table 2 exhibits the high CEM-101 potency against *Bacillus* spp. (MIC<sub>90</sub>, 0.03 μg/ml), *Corynebacterium* spp. (MIC<sub>90</sub>, 0.5 μg/ml), *L. monocytogenes* (MIC<sub>90</sub>, 0.03 μg/ml), Micrococcus spp. (MIC<sub>90</sub>, 0.03 μg/ml) and various other rarely isolated Gram-positive organisms (9 species). Only three (5.7%) strains had a CEM-101 MIC at >0.5 μg/ml.
- The collection of *N. gonorrhoeae* was selected to maximize resistances to penicillin (47.1%; ß-lactamase and chromosomal mechanisms), tetracyclines (32.4%) and fluoroquinolones (44.1% non-susceptible to ciprofloxacin).

CEM-101 (MIC<sub>90</sub>, 0.12 µg/ml) was four-fold more potent than azithromycin and equal to the activity of ceftriaxone. All gonococcal strains were inhibited by CEM-101 at ≤0.25 µg/ml.

The CEM-101 MIC results for 71 anaerobes are found in Table 4. CEM-101 was most active (MIC<sub>50</sub>, ≤0.03-0.12 μg/ml) against *Prevotella* spp., *Porphyromonas* spp., *C. difficile*, other *Clostridium* spp. and peptostreptococci. Gramnegative anaerobes (MIC<sub>90</sub>, 2-4 μg/ml; *B. fragilis* and group) were less CEM-101-susceptible. One-half of *C. difficile* strains were highly resistant to CEM-101. CEM-101 was two- to eight-fold more active than telithromycin against this collection of strict anaerobes (data not shown).

Table 1. Activity of CEM-101 and selected comparison agents tested against enterococci.

Organism/Resistance

(5 strains), and *E. raffinosus* (3 strains).

subset (no. tested)

Subset (110. testeu)	7 tittiiriioi obiai agoitt	0070	<b>30</b> 70	range
E. faecalis				
Vancomycin-susceptible (29)	CEM-101	0.03	2	0.015-2
	Telithromycin	0.06	4	0.03-8
	Erythromycin	2	>4	0.25-4
	Clarithromycin	2	>16	0.015->16
	Azithromycin	8	>16	0.5->16
	Quinupristin/dalfopristin	4	>4	1->4
	Levofloxacin	1	>4	0.25->4
	Linezolid	2	2	0.5-2
Vancomycin-resistant (10)	CEM-101	0.25	2	0.015-2
	Telithromycin	0.5	4	0.03-4
	Erythromycin	>4	>4	0.5->4
	Clarithromycin	16	>16	0.015->16
	Azithromycin	>16	>16	2->16
	Quinupristin/dalfopristin	4	>4	4->4
	Levofloxacin	>4	>4	0.25->4
	Linezolid	1	2	1-2
E. faecium		–		
Vancomycin-susceptible (30)	CEM-101	0.25	2	0.03-2
	Telithromycin	0.5	4	0.03-8
	Erythromycin	>4	>4	≤0.12->4
	Clarithromycin	>16	>16	0.06->16
	Azithromycin	>16	>16	0.25->16
	Quinupristin/dalfopristin	0.5	2	0.5-4
	Levofloxacin	>4	>4	1->4
	Linezolid	2	2	1-2
Vancomycin-resistant (10)	CEM-101	2	2	0.25-2
	Telithromycin	4	4	2-4
	Erythromycin	>4	>4	0.25->4
	Clarithromycin	>16	>16	0.15->16
	Azithromycin	>16	>16	>16
	Quinupristin/dalfopristin	0.5	1	0.5-1
	Levofloxacin	>4	>4	0.25->4
	Linezolid	1	2	1-2
Other enterococci (20)ª	CEM-101	0.03	1	0.015-2
<b>\</b> - /	Telithromycin	0.06	2	0.015-4
	Erythromycin	1	>4	≤0.12->4
	Clarithromycin	0.5	>16	0.03->16
	Azithromycin	2	>16	0.12->16
	Quinupristin/dalfopristin	2	2	0.5-4
	Levofloxacin	2	>4	0.25->4
	Linezolid	1	2	1-2

# Table 2. CEM-101 activity compared to 10 other agents when tested against uncommonly isolated Gram-positive species (53 strains).

MIC (µg/ml)

Bacillus spp.(10) <sup>a</sup>				
CEM-101	0.015	0.03	≤0.008-0.03	
Telithromycin	0.03	0.06	0.015-0.25	
Erythromycin	0.25	2	≤0.12-2	
Clarithromycin	0.12	0.25	0.06-0.5	
Azithromycin	2	4	0.25-4	
	4	-		
Clindamycin	1	4	0.5->4	
Quinupristin/dalfopristin	1	1	0.5-2	
Amoxicillin/clavulanate	4	>8	≤0.25->8	
Cefdinir	2	>4	≤0.12-0.25	
Levofloxacin	≤0.12	0.25	≤0.12-0.25	
Linezolid	1	1	1-2	
Trimethoprim/sulfamethoxazole	0.5	1	≤0.25-2	
mmethophim/sunamethoxazoie	0.5	ı	≥0.25-2	
Corynebacterium spp. (10)b				
CEM-101	0.015	0.5	≤0.008-16	
	0.06	8	≤0.008->16	
Telithromycin		_		
Erythromycin	>4	>4	≤0.12->4	
Clarithromycin	8	>16	0.015->16	
Azithromycin	>16	>16	0.12->16	
Clindamycin	>4	>4	1->4	
Quinupristin/dalfopristin	0.25	1	≤0.12-1	
Amoxicillin/clavulanate	>8	>8	0.5->8	
Cefdinir	>4	>4	0.25->4	
		_		
Levofloxacin	>4	>4	0.25->4	
Linezolid	0.25	0.5	0.25-0.5	
Trimethoprim/sulfamethoxazole	>4	>4	≤0.25->4	
(40)				
L. monocytogenes (10)				
CEM-101	0.03	0.03	0.03	
Telithromycin	0.06	0.06	0.06	
Erythromycin	0.25	0.25	0.25	
Clarithromycin	0.12	0.12	0.12	
Azithromycin	0.5	1	0.5-1	
•		7		
Clindamycin	2	2	1-4	
Quinupristin/dalfopristin	2	2	1-2	
Amoxicillin/clavulanate	0.5	0.5	≤0.25-1	
Cefdinir	>4	>4	4->4	
Levofloxacin	1	1	1	
Linezolid	2	2	2	
		≥ ≤0.25		
Trimethoprim/sulfamethoxazole	≤0.25	≥0.25	≤0.25	
Micrococcus spp. (10)°				
CEM-101	0.015	0.03	≤0.008-0.06	
Telithromycin	0.03	0.06	0.015-0.12	
Erythromycin	≤0.12	0.5	≤0.12-2	
Clarithromycin	0.06	0.5	0.03-2	
Azithromycin	0.12	0.5	0.06-4	
Clindamycin	0.25	0.25	≤0.12-2	
Quinupristin/dalfopristin	≤0.12	0.5	≤0.12-0.5	
Amoxicillin/clavulanate	≤0.25	≤0.25	≤0.25-0.5	
Cefdinir	0.5	0.5	≤0.12-0.5	
Levofloxacin	1	1	1	
Linezolid	1	1	0.5-1	
Trimethoprim/sulfamethoxazole	≤0.25	≤0.25	≤0.25	
•				
Other species (13)d				
CEM-101	0.03	2	≤0.008-8	
Telithromycin	0.12	8	≤0.0008-8	
Erythromycin	0.5	>4	≤0.12->4	
Clarithromycin	0.25	>16	0.015->16	
•				
Azithromycin	0.5	>16	0.015->16	
Clindamycin	≤0.12	>4	≤0.12->4	
Quinupristin/dalfopristin	0.5	1	0.25->4	
Amoxicillin/clavulanate	≤0.25	>8	≤0.25->8	
Cefdinir	0.5	>4	≤0.12->4	
Levofloxacin	1	2	≤0.12->4	
Linezolid	1	2	≤0.12 <b>-</b> >4 ≤0.12->4	
	1			
Trimethoprim/sulfamethoxazole	I	>4	≤0.25->4	
a. Includes: <i>Bacillus cereus</i> (8 strains), <i>B. ci</i>	irculans (1 strain) and	R megaterium (1 etr	ain)	
a. Indiados. Dadinas dorbas (O sulanis), D. G	rodiano (i odalin, and	mogatoriam (1 stic	۵11 1/1	

- a. Includes: Bacillus cereus (8 strains), B. circulans (1 strain), and B. megaterium (1 strain).
   b. Includes: Corynebacterium jeikeium (5 strains), C. striatum (3 strains), C. urealyticum (1 strain) and C.
- xerosis (1 strain).
  c. Includes: *Micrococcus luteus* (2 strains), and unspeciated *Micrococcus* (8 strains).
- d. Includes: Aerococcus viridans (1 strain), Bordetella bronchiseptica (1 strain), Dermabacter hominis (1 strain), Gemella morbillorum (1 strain), Lactococcus lactis (1 strain), Rothia mucilaginosa (3 strains), Weissella confusa (1 strain), unspeciated Aerococcus (3 strains), and unspeciated Stomatococcus (1 strain).

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Range

				M	IC (µg/ml)					9	by category	/ <sup>:a</sup>	
Antimicrobial agent	50%			90%				Range		Susceptible/Resi			
CEM-101		0.06			0.12		(	0.03-0.25		-/-			
Azithromycin		0.25			0.5				-/-				
Ceftriaxone		≤0.015			0.12		≤0.015-0.25				100.0 / -		
Ciprofloxacin		0.008			>32	≤0.002->32				55.9 / 14.7			
Penicillin		1		32			<u>≤</u>	≤0.015-64			23.5 / 47.1		
Tetracycline	1				0.03-16				23.5 / 32.4				
a. Criteria as published by the CLSI [200	7].												
Table 4. CEM-101 MIC species (224		ons for s	selected	Gram-p	ositive (	organism	ıs, <i>N. g</i> o	norrhoe	eae and a	all tested	l anaero	bic	
Occurrences at N													
Organism (no. tested)/group	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16	
E. faecalis (39)	0	4	13	2	0	5 <sup>a</sup>	<b>4</b> a	<b>4</b> a	<b>7</b> a	0	0	0	
E. faecium (40)	0	0	13	1	0	<b>3</b> a	0	<b>7</b> a	16 <sup>a</sup>	0	0	0	
Bacillus spp. (10)	3	4	3	0	0	0	0	0	0	0	0	0	

CEM-101 tosted against 34 isolates of N. gonorrhogae having high rates of resistance to fluorequinclenes

							Occurrences a	at MIC (µg/m	l)				
Organism (no	. tested)/group	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
E. faecalis (39	9)	0	4	13	2	0	5 <sup>a</sup>	<b>4</b> a	<b>4</b> a	<b>7</b> a	0	0	0
E. faecium (40	0)	0	0	13	1	0	3 <sup>a</sup>	0	<b>7</b> a	16 <sup>a</sup>	0	0	0
Bacillus spp. (	(10)	3	4	3	0	0	0	0	0	0	0	0	0
Corynebacter	ium spp. (10)	3	3	0	1	1	0	1	0	0	0	0	1
Listeria spp. (	10)	0	0	10	0	0	0	0	0	0	0	0	0
Micrococcus	spp. (10)	2	4	3	1	0	0	0	0	0	0	0	0
N. gonorrhoea	ae (34)	0	0	4	20	9	1	0	0	0	0	0	0
B. fragilis (10)		_b	-	0	0	0	0	0	2	1	4	1	2
B. fragilis grou	up (11)	-	-	0	0	0	0	1	0	5	1	0	4
Prevotella spp	o. (10)	-	-	1	1	3	2	0	0	1	1	0	1
Porphyromon	as spp. (10)	-	-	8	2	0	0	0	0	0	0	0	0
C. difficile (10	)	-	-	0	2	3	0	0	0	0	0	0	5
Clostridium sp	pp. (10)	-	-	4	5	0	0	0	0	0	0	0	1
Peptostreptod	occus spp. (10)	-	-	3	5	0	2	0	0	0	0	0	0

a. Genarally macrolide-resistant.b. -= untested MIC.

#### CONCLUSIONS

- CEM-101 demonstrated excellent activity against
  - Macrolide-susceptible enterococci (MIC<sub>90</sub>, 0.25 μg/ml)
  - *Bacillu*s spp. (MIC<sub>90</sub>, 0.03 μg/ml)
  - Corynebacterium spp. (MIC<sub>90</sub>, 0.5 µg/ml)
  - *L. monocytogenes* (MIC<sub>90</sub>, 0.03 μg/ml)
  - *Micrococcus* spp. (MIC<sub>90</sub>, 0.03 μg/ml)
- CEM-101 exhibited potent inhibition (MIC<sub>90</sub>, 0.12 μg/ml) of gonococci, including ciprofloxacin-resistant clinical strains from the USA. Clinical trials should be considered.
- CEM-101 inhibited strict anaerobes, especially Grampositive species at ≤0.25 μg/ml.
- CEM-101 may be suited for wider clinical applications including STD indications.

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