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# Antimicrobial Characterization of CEM-101: Single Step, Selection by Passaging and Inducible Resistances

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## ABSTRACT

**Background:** CEM-101, an orally administered macrolide-ketolide for respiratory tract infections (RTI), has potent activity against Gram-positive pathogens, *H. influenzae* and *M. catarrhalis*. To further define resistance (R) potential to CEM-101, 3 studies determined single step mutational rates, passaging selection and R induction by erythromycin (ERY).

**Methods:** Single step R used 1 *S. aureus* (SA), 1 *E. faecalis*, 2 *S. pneumoniae* (SPN), exposed to 4X, 8X and 16X MIC of CEM-101. Selection by passaging (7 days), used subinhibitory concentrations of CEM-101 and 3 comparators (azithromycin, clarithromycin, telithromycin [TEL]) with 18 strains including SA, CA-MRSA USA300, enterococci and SPN with various ERY-R patterns. Induction experiments with D-test (ERY inducer + CEM-101, clindamycin [CC] and TEL) tested 81 ERY-R, CC-S strains (17 spp).

**Results:** In R selection passaging, no significant variation was observed for 8 strains (44.4%; 4 spp). The remaining 10 strains exhibited modest CEM-101 MIC increases of 4- (7 strains) or 8-fold (3) without reversion of the MIC in drug-free media. R-selection during passaging was less for CEM-101 compared to other agents evaluated. No CEM-101 single-step mutations were observed at 4X, 8X or 16X CEM-101 MIC using inocula of 6.5 X $10^8$ (SPN) to 6.0 X $10^9$ (SA; see Table). Four patterns of ERY induction of CEM-101/TEL/CC-R were noted as follows: +/+/+ (39; 10 spp, *erm* A, B and C); -/+/+ (7; 2 spp, *erm* A); +/+/- (10; 4 spp, *msr* A) and -/-/- (25; 10 spp, none).

**Table. Results of the single-step mutation studies.**

| Organism | Single step mutation rate[a] |
|---|---|
| *E. faecalis* ATCC 29212 | <4.0 X $10^{-9}$ |
| *S. aureus* ATCC 29213 | <6.0 X $10^{-9}$ |
| *S. pneumoniae* 063-1085A (wild-type) | <1.4 X $10^{-9}$ |
| *S. pneumoniae* 075-241B (ermB) | <6.5 X $10^{-8}$ |

a. Strains were exposed at 4X, 8X and 16X CEM-101 MIC.

**Conclusions:** CEM-101 propensity for R was considered low for single step at <$10^{-8}$ or $10^{-9}$; infrequent by selection (passaging) and induction was comparable to CC but less than TEL. CEM-101 warrants further consideration as a RTI treatment agent.

## INTRODUCTION

CEM-101 is an orally-administered macrolide-ketolide agent with a preliminary in vitro spectrum most similar to telithromycin. Therefore, this novel agent has possible clinical applications against respiratory tract infections (CA-RTI) and skin and skin structure infections (SSSI) caused by Gram-positive (*Staphylococcus aureus*, *Streptococcus* spp., enterococci) and fastidious Gram-negative species (*Haemophilus influenzae*). To determine the probability of resistance emergence on CEM-101 therapy and/or spontaneous/inducible resistance occurring, we tested selected organisms representing wild type (WT) and documented resistant populations of pathogens likely to be isolated from CA-RTI or SSSI.

## MATERIALS AND METHODS

Resistance induction by erythromycin (ER):
The CLSI disk diffusion method (M2-A9, 2006) was utilized to D-test staphylococci (on Mueller-Hinton [MH] agar) and streptococci (on MH agar with 5% sheep blood). ER was used as the inducing agent (Figure 1) with clindamycin (disk code, CC), telithromycin (disk code, TEL) and CEM-101 disks placed around the ER inducer (disk code, E) at distances of 12 and 15 mm for *Streptococcus* spp. and staphylococci, respectively.

Organisms to be tested include previously confirmed D-test-positive and –negative control isolates in each group of strains having the ER-resistant, clindamycin- susceptible phenotype.

- Staphylococci (31 strains total; 21 D-test-positive/10 D-test-negative with clindamycin substrate): *S. aureus* (15/5) and coagulase-negative staphylococci (6/5)
- Streptococci (50 strains total; 25 D-test-positive/25 D-test-negative): *S. pneumoniae* (6/14), ß-haemolytic streptococci (15/5), and viridans group streptococci (4/6)

CLSI quality control (QC) strains, *S. aureus* ATCC 25923, ATCC BAA-977, ATCC BAA-976, *S. pneumoniae* ATCC 49619, *S. pyogenes* ATCC 19615, and *S. agalactiae* ATCC 12386, were also tested. Selected organisms (D-test positive) were screened by PCR molecular methods for macrolide resistance mechanisms (see Tables 2 and 3).

Passaging and single-step mutational studies:
Reference broth microdilution method according to the CLSI document M7-A7 (2006) and interpretive criteria published by the CLSI (M100-S18, 2008) were used. CEM-101 was tested in a 12 $\log_2$ dilution schedule using MH broth medium ± lysed horse blood supplement. Azithromycin, clarithromycin and telithromycin were tested as control drugs.

Passaging was performed by removing the entire content of the last well with growth into broth media. After growth up to an adequate bacterial suspension (0.5 McFarland), the proper diluted suspension (5 x $10^5$ CFU/ml) was transferred to testing MIC panels and repeated through seven passage days. Reversion to susceptible was assessed by three consecutive passages performed on drug-free agar with final MIC retest by the broth microdilution method.

For single-step mutation rate studies, fresh colonies from an agar plate were emulsified in sterile broth until approximately a 4 McFarland turbidity standard (target concentration of 1.2 X $10^9$ CFU/ml) was achieved. An aliquot suspension was plated on appropriate agar plates containing 4X, 8X and 16X CEM-101 MIC. Serial dilutions of the inoculum suspension were plated on antimicrobial-free agar plates to determine the colony count (CFU/ml).

A total of 18 strains were tested for resistance selection during passaging in subinhibitory concentrations of CEM-101 and to peer drugs, including six *S. aureus*, one coagulase-negative staphylococci, five enterococci, four *S. pneumoniae*, one ß-haemolytic streptococci and one viridans group streptococci (see Tables 2 and 3). Nine additional strains were evaluated for resistance selection rates for CEM-101 only, and the following isolates were evaluated for single-step mutation events:

- Macrolide-susceptible *S. aureus* (ATCC 29213)
- Macrolide-susceptible *Enterococcus faecalis* (ATCC 29212)
- Macrolide-susceptible *S. pneumoniae* (063-1085A)
- Macrolide-resistant, CEM-101-susceptible *S. pneumoniae* (*erm*B; 075-241B)

## RESULTS

- For *S. aureus* strains, ER induced clindamycin resistance in 15 of 20 (75%) tested isolates, however telithromycin and CEM-101 showed 100% induction. Inducible resistance by ER to all three agents is illustrated in Figure 1.
- Among the CoNS, six strains had ER-induced resistance to all three agents, but five strains (three *S. epidermidis*, one *S. capitis* and one *S. haemolyticus*) did not demonstrate resistance to clindamycin only.
- Three patterns of erythromycin inducible resistance were noted among the ß-haemolytic streptococci: 1.) ER inducible resistance to all drugs (eight occurrences); 2.) ER inducible resistance for clindamycin and telithromycin but not CEM-101 (seven occurrences); 3.) no ER induction of resistances (five occurrences).
- Among 18 isolates tested for resistance selection during passaging, no significant variation (more than one $\log_2$ dilution) of the MIC values of CEM-101 were observed with eight strains (44.4%). The remaining 10 strains exhibited modest increases of CEM-101 MIC values of four- or eight-fold, with no reversion or only two-fold decrease in the MIC after three subcultures on antimicrobial free media (Table 2).
- No growth of resistant mutants was observed when four strains were exposed to 4X, 8X and 16X CEM-101 MIC. The mutation rates by organism were: *E. faecium* at <4.0 X $10^{-9}$, *S. aureus* at <6.0 X $10^{-9}$ and *S. pneumoniae* at <6.5 X $10^{-8}$ to <1.4 X $10^{-9}$ (Table 3).

**Table 1. Patterns of inducible CEM-101, telithromycin and clindamycin resistance by erythromycin using a modified D-test method.**

| Induced resistance to: | | | No. occurrences: | |
|---|---|---|---|---|
| Clindamycin | Telithromycin | CEM-101 | Staphylococci (31) | Streptococci (50) |
| + | + | + | 21[a] | 18 |
| - | + | + | 10 | 0 |
| + | + | - | 0 | 7 |
| - | - | - | 0 | 25 |

a. See Figure 1.

**Table 2. Results of serial passaging experiments.**

| Organism/Antimicrobial | Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Reversion |
|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* ATCC 29213 | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.12 | 0.25 | 0.12 | 0.12 | 0.12 | 0.12 |
| Telithromycin | 0.12 | 0.12 | 0.5 | 0.25 | 0.12 | 0.12 | 0.12 | 0.25 | 0.12 |
| Clarithromycin | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 |
| Azithromycin | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| *S. haemolyticus* 064-4090A (*erm*A) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.12 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 |
| Telithromycin | 0.12 | 0.25 | 0.25 | 0.25 | 0.5 | 1 | 1 | 1 | 1 |
| *E. faecalis* ATCC 29212 | | | | | | | | | |
| CEM-101 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Telithromyci | 0.06 | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.06 | 0.06 |
| Clarithromycin | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 |
| Azithromycin | 16 | 8 | 16 | 8 | 8 | 8 | 8 | 8 | 8 |
| *S. pneumoniae* ATCC 49619 | | | | | | | | | |
| CEM-101 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.03 | 0.03 | 0.015 |
| Telithromycin | 0.015 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.03 |
| Clarithromycin | 0.03 | 0.03 | 0.03 | 0.06 | 0.03 | 0.03 | 0.06 | 0.06 | 0.03 |
| Azithromycin | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| *S. pneumoniae* 063-1085A (wild-type) | | | | | | | | | |
| CEM-101 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.03 | 0.03 | 0.06 | 0.06 |
| Telithromycin | 0.015 | 0.015 | 0.015 | 0.03 | 0.03 | 0.03 | 0.03 | 0.12 | 0.06 |
| Clarithromycin | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.12 | 0.06 |
| Azithromycin | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.12 |
| *S. pneumoniae* 075-241B (*erm*B) | | | | | | | | | |
| CEM-101 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.03 |
| Telithromycin | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.06 | 0.06 | 0.03 |
| *S. pneumoniae* 127-2273B (*mef*A) | | | | | | | | | |
| CEM-101 | 0.06 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| Telithromycin | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Clarithromycin | 8 | 8 | 4 | 8 | 8 | 8 | 8 | 8 | 8 |
| Azithromycin | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| *S. pyogenes* 117-1612A (wild-type) | | | | | | | | | |
| CEM-101 | 0.015 | 0.015 | 0.015 | 0.015 | 0.03 | 0.03 | 0.03 | 0.015 | 0.015 |
| Telithromycin | 0.015 | 0.015 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Clarithromycin | 0.03 | 0.03 | 0.03 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.03 |
| Azithromycin | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 |
| *S. mitis* 051-4933A (*mef*A) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 |
| Telithromycin | 0.5 | 1 | 0.5 | 0.5 | 1 | 1 | 1 | 2 | 2 |
| Clarithromycin | 2 | 4 | 4 | 4 | 4 | 8 | 8 | 16 | 8 |
| Azithromycin | 4 | 4 | 8 | 8 | 8 | 16 | 16 | 16 | 16 |
| *S. aureus* 707J (USA300-0114) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.25 | 0.5 | 0.5 | 0.5 | 1 | 0.5 |
| *S. aureus* 004-573D (USA300) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.25 | 0.5 | 0.5 | 0.5 | 1 | 0.5 |
| *S. aureus* 117-472D (USA300) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| *S. aureus* 024-11490A (USA300) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| *S. aureus* 117-453D (USA300) | | | | | | | | | |
| CEM-101 | 0.12 | 0.12 | 0.12 | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 | 0.25 |
| *E. faecalis* 061-6556A (Ery-S)[a] | | | | | | | | | |
| CEM-101 | 0.06 | 0.06 | 0.06 | 0.12 | 0.12 | 0.12 | 0.25 | 0.25 | 0.06 |
| *E. faecalis* 067-6633A (*erm*B) | | | | | | | | | |
| CEM-101 | 2 | 4 | 4 | 4 | 8 | 8 | 8 | 16 | 8 |
| *E. faecium* 067-1457A (Ery-S)[a] | | | | | | | | | |
| CEM-101 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| *E. faecium* 086-15387A (*erm*B) | | | | | | | | | |
| CEM-101 | 1 | 1 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |

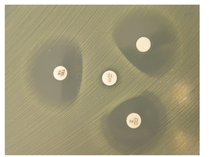
a. Ery-S = erythromycin-susceptible.

**Table 3. Results of the single-step mutation studies.**

| Organism | Single step mutation rate[a] |
|---|---|
| *E. faecalis* ATCC 29212 | <4.0 X $10^{-9}$ |
| *S. aureus* ATCC 29213 | <6.0 X $10^{-9}$ |
| *S. pneumoniae* 063-1085A (wild-type) | <1.4 X $10^{-9}$ |
| *S. pneumoniae* 075-241B (*erm*B) | <6.5 X $10^{-8}$ |

a. Strains were exposed at 4X, 8X and 16X CEM-101 MIC.

**Figure 1. Erythromycin (E15) induction of resistances to clindamycin (CC2), telithromycin (TEL15) and CEM-101 (blank); *S. aureus*.**



## CONCLUSIONS

- ER demonstrated the ability to induce resistance to clindamycin, telithromycin and CEM-101 (less than telithromycin). Like currently available $MLS_B$-ketolide compounds, CEM-101 use for serious Gram-positive infections (bacteremia, endocarditis, osteomyelitis) may require D-test results to assist optimal patient management.
- Results of resistance selection during passaging in subinhibitory concentrations were less overall for CEM-101 when compared to other agents evaluated such as telithromycin.
- No CEM-101-resistant mutants were observed at 4X, 8X or 16X CEM-101 MIC using inoculum concentrations of 6.5 X $10^8$ (*S. pneumoniae*) to 6.0 X $10^9$ (*S. aureus*) CFU/ml.

## SELECTED REFERENCES

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