

ACTIVITY OF CEM-101 COMPARED TO OTHER AGENTS AGAINST MACROLIDE SUSCEPTIBLE AND -RESISTANT STREPTOCOCCI

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Revised Abstract

Background: Strains of *S. pneumoniae* resistant to β-lactams, macrolides, quinolones and other agents occur worldwide and resistant non-vaccine serotypes have appeared. CEM-101 is member of the macrolide-ketolide group which is 2-4 times more active than tetracycline against macrolide resistant pneumococci. Infections caused by drug-resistant group A streptococci are encountered worldwide and sometimes life-threatening infections caused by these organisms are encountered. Streptococcus pyogenes strains, although retaining their β-lactam susceptibility are sometimes macrolide resistant. Tetracycline is active against all macrolide resistant *S. pyogenes* genotypes except for *erm(B)*. We tested 1) activity of CEM-101 compared to those of erythromycin, azithromycin, clarithromycin, tetracycline, clindamycin, penicillin G, amoxicillin/clavulanate, levofloxacin, and moxifloxacin against a range of pneumococci; 2) potency of CEM-101, erythromycin, azithromycin, clarithromycin, tetracycline, clindamycin, penicillin G, amoxicillin/clavulanate, levofloxacin and moxifloxacin against 124 *S. pyogenes* strains. **Methods:** The 221 pneumococcal clinical isolates included 50 macrolide susceptible and 171 macrolide resistant strains. Of these 53 were penicillin G susceptible, 63 intermediate and by 105 penicillin resistant by old CLSI breakpoints; 27 strains were quinolone resistant with defined QDR mutations. Macrolide resistant strains comprised 54 with *erm(B)*, 51 *mei(A)*, 4 *erm(A)*, 31 *erm(B)* + *mei(A)*, 127 with L4, and 4 with 23S rRNA ribosomal protein mutations. The group A streptococci comprised 26 macrolide susceptible and 98 macrolide resistant organisms (19 *erm(B)*, 38 *mei(A)*, 40 *erm(A)*, 1 strain with L4 ribosomal protein mutation). Agar dilution with Mueller-Hinton agar + 5% sheep blood with inocula of 10⁷ cfu/spot was used. Plates were incubated overnight in air for 35°C with usual quality controls. **Results:** Pneumococcal MIC₅₀ and MIC₉₀ values (μg/ml) were as follows:

Drug	Macrolide susceptible (50)			Macrolide resistant (171)		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
CEM-101	0.002-0.015	0.008	0.015	0.006-1	0.06	0.25
Erythromycin	0.002-0.25	0.06	0.125	0.004-1	0.06	0.25
Azithromycin	0.004-0.25	0.125	0.125	0.004-1	0.06	0.25
Clarithromycin	0.002-0.03	0.03	0.06	0.004-0.125	0.03	0.06
Tetracycline	0.015-0.03	0.03	0.03	0.002-0.125	0.03	0.06
Clinda	0.015-0.06	0.03	0.06	0.004-0.125	0.03	0.06
Pen G	0.015-1	1	2	0.008-18	1	4
Amox	0.125-1	0.125	0.125	0.002-2	0.125	0.25
Moxi	0.125-8	0.25	4	0.125-8	0.25	0.5

MICs for group A streptococci were as follows:

Drug	Macrolide susceptible (26)			Macrolide resistant (98)		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
CEM-101	0.008-0.03	0.015	0.03	0.015-1	0.06	0.5
Erythromycin	0.002-0.25	0.06	0.125	0.004-1	0.06	0.25
Azithromycin	0.004-0.25	0.125	0.125	0.004-1	0.06	0.25
Clarithromycin	0.002-0.03	0.03	0.06	0.004-0.125	0.03	0.06
Tetracycline	0.002-0.03	0.03	0.06	0.004-0.125	0.03	0.06
Clinda	0.015-0.06	0.03	0.06	0.004-0.125	0.03	0.06
Amoxicillin	0.015-0.03	0.03	0.06	0.004-0.125	0.03	0.06
Pen G	0.008-0.125	0.015	0.015	0.008-125	0.015	0.015
Moxi	0.125-0.25	0.25	0.25	0.008-125	0.25	0.25

CEM-101 had an MIC range against macrolide susceptible pneumococci of 0.002-0.015 μg/ml and a range against macrolide resistant pneumococci (all phenotypes) of 0.004-1 μg/ml. Only 3 strains with *erm(B)* [with and without *mei(A)*] had CEM-101s MIC of 1.0 μg/ml and 218/221 strains had CEM-101 MICs of ≤0.5 μg/ml.

Revised Abstract cont'd

By contrast corresponding tetracycline MIC ranges were 0.015-0.03 μg/ml for macrolide susceptible and 0.03-2 μg/ml for macrolide resistant strains, respectively. CEM-101 MICs were up to four fold lower than those of tetracycline against macrolide susceptible and resistant strains. MICs of erythromycin, azithromycin, clarithromycin were highest in *erm(B)* [with and without *mei(A)*], L4 and 23S rRNA strains and clindamycin resistance only seen amongst strains containing *erm(B)* with or without *mei(A)*. All strains were β-lactam and quinolone susceptible. Against group A streptococci, CEM-101 MICs ranged between 0.008-0.03 μg/ml against macrolide susceptible, and between 0.015-1 μg/ml against macrolide resistant strains. Against *erm(B)* strains, erythromycin, azithromycin, clarithromycin MICs were 32-64 μg/ml while 17/19 strains had tetracycline MICs between 4 and 16 μg/ml, comparative CEM-101 MICs were 0.015-1 μg/ml. By comparison, *erm(A)* and *mei(A)* strains had CEM-101 MICs of 0.015-0.5 μg/ml, clindamycin and tetracycline MICs ≤1 μg/ml, with erythromycin, azithromycin and clarithromycin MICs of 0.5-64 μg/ml. **Conclusions:** CEM-101 had the lowest MICs of all macrolides and ketolides against all pneumococcal strains including macrolide resistant phenotypes. CEM-101 was very potent against all strains of group A streptococci tested irrespective of macrolide resistance phenotype.

Introduction

Strains of *Streptococcus pneumoniae* resistant to macrolides, β-lactams, quinolones, and other agents occur worldwide (4). Macrolide resistance, which is now predominant in some countries such as Japan and Korea, is most likely due to overuse of azithromycin and clarithromycin during the past 15 years. Macrolide resistance also usually occurs (although genetically unlinked) together with penicillin G resistance (4). Although all strains of group A streptococci are still β-lactam susceptible, macrolide resistance does occur, especially in southern, central and eastern Europe and Asia (6).

Although the pediatric conjugate vaccine has dramatically decreased meningitis and bacteremia caused by most of the usual drug-resistant pneumococcal clones, a recent paper by Pichichero and Casey (7) has described an outbreak of serious cases of otitis media caused by penicillin-resistant strains with a serotype (19A) not included in the vaccine. Thus the problem of drug-resistant pneumococci causing community-acquired respiratory infection, especially in children, is likely to worsen with the spread of this clone.

Introduction of tetracycline into the therapeutic armamentarium was, with the exception of *erm(B)* group A streptococci (which are naturally tetracycline resistant), serious cases of otitis media caused by penicillin-resistant strains with a serotype (19A) not included in the vaccine. Thus the problem of drug-resistant pneumococci causing community-acquired respiratory infection, especially in children, is likely to worsen with the spread of this clone.

CEM-101 is an experimental macrolide/ketolide which is 2-4 fold more active than tetracycline. In the current study we have performed MIC studies to compare the activity of CEM-101 to that of erythromycin, azithromycin, clarithromycin, tetracycline, clindamycin, penicillin G, amoxicillin/clavulanate, levofloxacin and moxifloxacin against a spectrum of pneumococci and group A streptococci with different macrolide resistance phenotypes and genotypes.

Materials and Methods

We tested 221 clinical pneumococcal strains: These comprised 50 macrolide susceptible and 171 macrolide resistant organisms. Macrolide resistant strains all had defined genotypes and comprised strains with *erm(B)* (54 strains), *mei(A)* (51 strains), *erm(B)* + *mei(A)* (31 strains), *erm(A)* (4 strains) and mutations in L4 (27 strains), and 23S rRNA ribosomal proteins (4 strains). These 221 strains also comprised 27 quinolone resistant phenotypes with defined QDRs (levofloxacin MICs ≥4 μg/ml) and the entire spectrum of penicillin G resistance phenotypes using the current CLSI penicillin G susceptibility classification. The 101 group A streptococci comprised 26 macrolide susceptible and 75 macrolide resistant strains. The latter comprised 13 strains with *erm(B)*, 24 *mei(A)*, 37 *erm(A)* and 1 strain with an L4 ribosomal protein mutation. MICs were done by the agar dilution technique which, although not specifically recommended by CLSI, has been used in our research laboratory for >20 years (3,5,6). Mueller-Hinton agar + 5% sheep blood agar was used, with 10⁷ cfu/spot and overnight incubation at 35°C in ambient air. The usual quality control strains were included in each run (2). CEM-101 was obtained from Cempra Pharmaceuticals and other drugs from either their respective manufacturers or Sigma Chemical, Inc.

TABLE 1. MICs (μg/ml) of drugs against pneumococcal strains

Drug	MIC range	MIC50	MIC90
Penicillin G (221)*	0.008-16	0.06	0.25
Penicillin G (53)	0.008-0.06	0.03	0.06
Penicillin G (19)	0.015-0.06	0.03	0.06
Penicillin R (105)	3-16	4	8
Moxifloxacin S (50)	0.002-0.5	0.2	0.5
erm(B)	0.015-16	0.06	1
mei(A)	0.008-4	0.125	0.4
erm(B) + mei(A)	0.015-0.03	0.03	0.06
erm(A)	0.015-8	0.5	2
L4	0.06-0.25	0.125	0.25
23S rRNA (4)	0.03-0.06	0.03	0.06
Quinolone S (195)	0.008-0.16	0.4	1
Quinolone R (27)	0.015-0.5	0.25	0.5
CEM-101	0.002-1	0.03	0.25
Erythromycin S	0.002-0.25	0.03	0.06
Erythromycin R	0.002-0.25	0.03	0.25
Penicillin R	0.004-1	0.03	0.25
Moxifloxacin S	0.002-0.015	0.008	0.015
erm(B)	0.004-1	0.03	0.25
mei(A)	0.008-0.15	0.03	0.125
erm(A)	0.008-0.015	0.03	0.125
erm(B) + mei(A)	0.015-1	0.125	0.25
L4	0.015-0.125	0.06	0.125
23S rRNA	0.002-0.03	0.03	0.06
Quinolone S	0.002-0.03	0.03	0.06
Quinolone R	0.004-1	0.038	0.25
Rifampin/S (4)	0.03-64	64	64
Penicillin R	0.015-64	64	64
Moxifloxacin S	0.002-0.015	0.008	0.015
erm(B)	0.004-1	0.03	0.25
mei(A)	0.008-0.15	0.03	0.125
erm(A)	0.008-0.015	0.03	0.125
erm(B) + mei(A)	0.015-1	0.125	0.25
L4	0.015-0.125	0.06	0.125
23S rRNA	0.002-0.03	0.03	0.06
Quinolone S	0.002-0.03	0.03	0.06
Quinolone R	0.004-1	0.038	0.25
Rifampin/S (4)	0.03-64	64	64
Penicillin R	0.015-64	64	64
Moxifloxacin S	0.002-0.015	0.008	0.015
erm(B)	0.004-1	0.03	0.25
mei(A)	0.008-0.15	0.03	0.125
erm(A)	0.008-0.015	0.03	0.125
erm(B) + mei(A)	0.015-1	0.125	0.25
L4	0.015-0.125	0.06	0.125
23S rRNA	0.002-0.03	0.03	0.06
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Quinolone R	0.004-1	0.038	0.25
Rifampin/S (4)	0.03-64	64	64
Penicillin R	0.015-64	64	64
Moxifloxacin S	0.002-0.015	0.008	0.015
erm(B)	0.004-1	0.03	0.25
mei(A)	0.008-0.15	0.03	0.125
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Rifampin/S (4)	0.03-64	64	64
Penicillin R	0.015-64	64	64
Moxifloxacin S	0.002-0.015	0.008	0.015
erm(B)	0.004-1	0.03	0.25
mei(A)	0.008-0.15	0.03	0.125
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