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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 ß-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 telithromycin 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 ß-lactam susceptibility are sometimes macrolide resistant. Telithromycin 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, telithromycin, clindamycin, penicillin G, amoxicillin/clavulanate, levofloxacin, and moxifloxacin against a range of pneumococci; 2) potency of CEM-101, erythromycin, azithromycin, clarithromycin, telithromycin, 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 QRDR mutations. Macrolide resistant strains comprised 54 with *erm*(B), 51 *mef*(A), 4 *erm*(A), 31 *erm*(B) + *mef*(A), 27 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 *mef*(A), 40 *erm*(A), 1 strain with L4 ribosomal protein mutation]. Agar dilution with Mueller-Hinton agar + 5% sheep blood with inocula of 10^4 cfu/spot was used. Plates were incubated overnight in air for 35ºC with usual quality controls. **Results**.Pneumococcal MIC_{50} and MIC_{90} 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	0.004-1	0.06	0.25
Erythro	0.03-0.25	0.06	0.125	1->64	>64	>64
Azithro	0.06-0.25	0.125	0.125	1->64	>64	>64
Clarithro	0.015-0.06	0.03	0.06	0.25->64	32	>64
Telithro	0.015-0.03	0.03	0.03	0.03-2	0.125	0.5
Clinda	0.015-0.06	0.03	0.06	0.03->64	0.125	>64
Amox/clav	0.015-8	0.5	2	0.015-16	1	8
Pen G	0.015-8	1	2	0.008->16	1	4
Levo	1-32	1	16	0.06-32	1	2
Moxi	0.125-8	0.25	4	0.125-4	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
Erythro	0.03-0.25	0.06	0.125	2->64	16	>64
Azithro	0.06-0.25	0.125	0.25	0.5->64	8	>64
Clarithro	0.015-0.06	0.03	0.06	0.25->64	4	>64
Telithro	0.03-0.06	0.06	0.06	0.03-16	0.25	8
Clinda	0.03-0.125	0.06	0.06	0.03->64	0.125	>64
Amox/clav	0.015-0.03	0.03	<0.015-0.125	0.03	0.03	
Pen G	<0.008-0.125	0.015	0.015	<0.008-0.125	0.015	0.015
Levo	0.5-1	0.5	0.5	0.5-2	0.5	1
Moxi	0.125-0.25	0.25	0.25	0.125-0.5	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 *mef*(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 telithromycin 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 telithromycin against macrolide susceptible and resistant strains. MICs of erythromycin, azithromycin, clarithromycin were highest in *erm*(B) [with and without *mef*(A)], L4 and 23s rRNA strains and clindamycin resistance only seen amongst strains containing *erm*(B) with or without *mef*(A). All strains were ß-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 telithromycin MICs between 4 and 16 µg/ml; comparative CEM-101 MICs were 0.015-1 µg/ml. By comparison, *erm*(A) and *mef*(A) strains had CEM-101 MICs of 0.015-0.5 µg/ml , clindamycin and telithromycin 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, ß-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 ß-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 pan-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 telithromycin into the therapeutic armamentarium was, with the exception of *erm*(B) group A streptococci (which are naturally telithromycin resistant), intended to solve the problem of macrolide resistance in streptococci (5,6). However, toxicities have limited the clinical utility of this drug. . Additionally, when the free AUC/MIC of telithromycin against macrolide-resistant pneumococci even with low MICs was examined carefully it could be seen that the number was not significantly above 25: thus resistance was predicted to occur and this has indeed been the case as evidenced by recent publications (8).

CEM-101 is an experimental macrolide/ketolide which is 2-4 fold more active than telithromycin. In the current study we have performed MIC studies to compare the activity of CEM-101 to that of erythromycin, azithromycin, clarithromycin, telithromycin, 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), *mef*(A) (51 strains), *erm*(B) + *mef*(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 QRDRs (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 *mef*(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 in use in my research laboratory for >20 years (3,5,6). Mueller-Hinton agar + 5% sheep blood agar was used, with 10^4 cfu/spot and overnight incubation at 35ºC 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[a])	0.008->16	1	4
Penicillin S (53)	0.008-0.06	0.03	0.06
Penicillin I (63)	0.125-1	0.5	1
Penicillin R (105)	2->16	4	8
Macrolide S (50)	0.015-8	1	2
erm (B)	0.03-16	1	4
mef (A)	0.008-4	0.125	4
erm (A)	0.03-0.03	----	----
erm (B) + *mef* (A)	0.03-8	2	4
L4 (27)	1->16	4	16
23S rRNA (4)	0.015-0.5	----	----
Quinolone S (195)	0.008->16	1	4
Quinolone R (27)	0.015-8	0.25	4
CEM-101	0.002-1	0.03	0.25
Pen-Mac S	0.002-0.25	0.03	0.125
Penicillin I	0.002-0.25	0.03	0.25
Penicillin R	0.004-1	0.06	0.25
Macrolide S	0.002- 0.015	0.008	0.015
erm (B)	0.004-1	0.03	0.5
mef (A)	0.008-0.25	0.03	0.125
erm (A)	0.008-0.015	----	----
erm (B) + *mef* (A)	0.015-1	0.125	0.25
L4	0.03-0.125	0.06	0.125
23S rRNA	0.002-0.03	----	----
Quinolone S	0.002-1	0.03	0.25
Quinolone R	0.004-.25	0.008	0.06
Erythromycin	0.015->64	64	>64
Penicillin S	0.03->64	4	>64
Penicillin I	0.03->64	>64	>64
Penicillin R	0.03->64	>64	>64
Macrolide S	0.03-0.25	0.06	0.125
erm (B)	16->64	>64	>64
mef (A)	1->64	4	32
erm (A)	2-4	----	----
erm (B) + *mef* (A)	4->64	>64	>64
L4	4->64	>64	>64
23S rRNA	8->64	----	----
Quinolone S	0.03->64	>64	>64
Quinolone R	0.03->64	0.06	>64
Clindamycin	0.06->64	16	>64
Penicillin S	0.06->64	4	>64
Penicillin I	0.06->64	>64	>64
Penicillin R	0.06->64	>64	>64
Macrolide S	0.06-0.25	0.125	0.0125
erm (B)	>64->64	>64	>64
mef (A)	1->64	4	8
erm (A)	2-8	----	----
erm (B) + *mef* (A)	2->64	>64	>64
L4	2->64	>64	>64
23S rRNA	32->64	----	----
Quinolone S	0.06->64	>64	>64
Quinolone R	0.06->64	0.125	>64
Telithromycin	0.125->64	8	>64
Penicillin S	0.015->64	1	>64
Penicillin I	0.03->64	16	>64
Penicillin R	0.015->64	16	>64
Macrolide S	0.015-0.06	0.03	0.06
erm (B)	4->64	>64	>64
mef (A)	0.5-32	2	4
erm (A)	0.25-0.5	----	----
erm (B) + *mef* (A)	1->64	>64	>64
L4	1-32	16	32
23S rRNA	8-16	----	----
Quinolone S	0.015 ->64	16	>64
Quinolone R	0.015->64	0.03	>64

[a] Number of strains tested.

Drug	MIC range	MIC50	MIC90
Telithromycin	0.015-2	0.06	0.5
Penicillin S	0.015-1	0.06	0.25
Penicillin I	0.015-1	0.06	0.5
Penicillin R	0.015-2	0.125	0.5
Macrolide S	0.015-0.03	0.03	0.03
erm (B)	0.03-2	0.06	1
mef (A)	0.03-0.5	0.125	0.25
erm (A)	0.03-0.06	----	----
erm (B) + *mef* (A)	0.03-2	0.5	1
L4	0.06-0.25	0.125	0.25
23S rRNA	0.03-0.06	----	----
Quinolone S	0.015 -2	0.125	0.5
Quinolone R	0.015-1	0.03	0.125
Clindamycin	0.015->64	0.06	>64
Penicillin S	0.03->64	0.06	>64
Penicillin I	0.03->64	0.125	>64
Penicillin R	0.015->64	0.06	>64
Macrolide S	0.015-0.06	0.03	0.06
erm (B)	0.06->64	>64	>64
mef (A)	0.03-0.125	0.06	0.06
erm (A)	0.125-0.25	----	----
erm (B) + *mef* (A)	0.03->64	0.06	>64
L4	0.03-0.125	0.06	0.125
23S rRNA	0.03-1	----	----
Quinolone S	0.015->64	0.06	>64
Quinolone R	0.03-64	0.03	64
Amox/Clav	0.015-16	0.05	8
Penicillin S	0.015-0.125	0.03	0.06
Penicillin I	0.03-2	0.5	1
Penicillin R	0.125-16	2	8
Macrolide S	0.015-8	0.5	2
erm (B)	0.015-8	0.5	8
mef (A)	0.015-8	0.25	2
erm (A)	0.03-0.03	----	----
erm (B) + *mef* (A)	0.03-16	2	8
L4	0.125-8	4	8
23S rRNA	0.03-0.06	----	----
Quinolone S	0.015-16	1	8
Quinolone R	0.015-4	0.5	2
Levofloxacin	0.06-32	1	8
Penicillin S	0.06-32	1	16
Penicillin I	1-32	1	2
Penicillin R	0.5-16	1	2
Macrolide S	1-32	1	16
erm (B)	0.5-32	1	2
mef (A)	0.5-8	1	2
erm (A)	1-1	----	----
erm (B) + *mef* (A)	1-16	1	16
L4	0.5-16	1	2
23S rRNA	0.06-1	----	----
Quinolone S	0.06-2	1	2
Quinolone R	4-32	16	16
Moxifloxacin	0.125-8	0.25	2
Penicillin S	0.125-8	0.5	4
Penicillin I	0.125-4	0.25	0.5
Penicillin R	0.125-4	0.25	0.5
Macrolide S	0.125-8	0.25	4
erm (B)	0.125	0.25	0.5
mef (A)	0.125-4	0.25	0.5
erm (A)	0.25-0.5	----	----
erm (B) + *mef* (A)	0.125-2	0.25	0.5
L4	0.125-4	0.25	0.5
23S rRNA	0.25-0.5	----	----
Quinolone S	0.125-1	0.25	0.5
Quinolone R	0.5-8	4	4

TABLE 2. MICs (µg/ml) of drugs against group A streptococci

Drug	MIC Range	MIC_{50}	MIC_{90}
CEM – 101 (124[a])	0.008-1	0.06	0.5
Macrolide S (26)	0.008-0.03	0.015	0.03
erm (B) (19)	0.03-1	0.5	1
mef (A) (38)	0.06-0.25	0.125	0.25
erm (A) (40)	0.016-0.5	0.03	0.125
L4 (1)	0.06	----	----
Erythromycin	0.03->64	16	>64
Macrolide S	0.03-0.25	0.06	0.125
erm (B)	>64->64	>64	>64
mef (A)	8-32	16	32
erm (A)	2->64	4	>64
L4	2	----	----
Telithromycin	0.06->64	0.25	8
Macrolide S	0.06-0.25	0.125	0.25
erm (B)	>64->64	>64	>64
mef (A)	0.5-16	8	8
erm (A)	2->64	16	>64
L4	2	----	----
Clarithromycin	0.015->64	4	>64
Macrolide S	0.015-0.06	0.03	0.06
erm (B)	32->64	>64	>64
mef (A)	0.5-8	4	8
erm (A)	0.25->64	2	>64
L4	1	----	----
Telithromycin	0.03-16	0.125	8
Macrolide S	0.03-0.06	0.06	0.06
erm (B)	0.03-16	8	16
mef (A)	0.125-1	0.5	1
erm (A)	0.03-0.25	0.06	0.125
L4	0.06	----	----
Amox/Clav	<0.015-0.125	0.03	0.03
Macrolide S	<0.015-0.03	0.03	0.03
erm (B)	<0.015-0.125	<0.015	0.03
mef (A)	<0.015-0.125	0.015	0.06
erm (A)	<0.015-0.03	0.03	0.03
L4	0.03	----	----
Levofloxacin	0.5-2	0.5	1
Macrolide S	0.5-1	0.5	0.5
erm (B)	0.5-1	0.5	1
mef (A)	0.5-2	0.5	1
erm (A)	0.5-2	0.5	1
L4	0.5	----	----
Moxifloxacin	0.03-0.5	0.25	0.25
Macrolide S	0.125-0.25	0.25	0.25
erm (B)	0.125-0.25	0.25	0.25
mef (A)	0.25-0.5	0.25	0.25
erm (A)	0.125-0.5	0.25	0.25
L4	0.25	----	----
Pen G	<0.008-0.125	0.015	0.015
Macrolide S	0.008-0.015	0.015	0.015
erm (B)	<0.008-0.125	0.015	0.015
mef (A)	<0.008-0.125	0.015	0.03
erm (A)	<0.008-0.015	0.015	0.015
L4	0.015	----	----
Clindamycin	0.03->64	0.125	>64
Macrolide S	0.03-0.125	0.06	0.06
erm (B)	0.06->64	>64	>64
mef (A)	0.03-0.125	0.06	0.125
erm (A)	0.06-0.5	0.125	0.25
L4	0.06	----	----

[a] Number of strains tested

Results

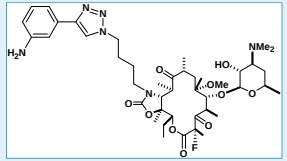
Results of pneumococcal MIC testing are presented in Table 1. CEM-101 had an MIC range against macrolide susceptible pneumococci of 0.002-0.016 µg/ml and a range against macrolide resistant pneumococci (all phenotypes) of 0.004-1 µg/ml. Only 3 strains with *erm*(B) [with and without *mef*(A)] had CEM-101s MIC of 1.0 µg/ml and 218/221 strains had CEM-101 MICs of ≤0.5 µg/ml. By contrast corresponding telithromycin MIC ranges were 0.016-0.03 µg/ml for macrolide susceptible and 0.016-2 µg/ml for macrolide resistant strains, respectively. CEM-101 MICs were up to four fold lower than those of telithromycin against macrolide susceptible and resistant strains. MICs of erythromycin, azithromycin, clarithromycin and clindamycin against the different macrolide resistant genotypes corresponds to what has been published before, with clindamycin resistance only seen amongst *erm*(B) strains (1,3,5).

All group A streptococcal strains were penicillin G susceptible. Against macrolide susceptible strains, CEM-101 MICs were 0.008-0.03 µg/ml and those against macrolide resistant strains (all phenotypes) MICs were 0.008-0.5 µg/ml. Telithromycin MICs were up to four fold higher than those of CEM-101. Importantly, 11/13 *erm*(B) strains were telithromycin resistant with MICs of 4 and 8 µg/ml (6) while all had low CEM-101 MICs, similar to those of other resistance phenotypes (range 0.016-0.5 µg/ml).

Conclusion

The potent activity of CEM-101 against all streptococcal strains tested irrespective of resistance phenotype points to a promising clinical future of this compound, subject to pharmacokinetic/pharmacodynamic, toxicity, and animal infection model studies.

Chemical Formula CEM-101



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