

# Antimicrobial Potenty and spectrum for Ro63-9141 (RO), a Novel Cephalosporin with Activity Against Methicillin-Resistant Staphylococci (MRS)

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### AMENDED ABSTRACT

**Background**: Community-acquired and nosocomial infections caused by multi-drug resistant Gram-positive (G+) pathogens are increasing. RO is a novel pyrrolidinone-3-ylidenemethyl cephem with high activity against MRS. In this study, RO was tested (NCCLS methods) against a large number of G+ isolates and respiratory tract pathogens.

Methods: Nearly all strains (1,703) were recent clinical isolates from the SENTRY Program (1997-2000) including 146 S. aureus (96 MRSA), 116 CoNS (90 MR), 128 Enterococcus spp., 519 S. pneumoniae (SPN), 85 viridans group streptococci (VgS), 103 ß-haemolytic strept (BHS), 418 H. influenzae (HI), 186 M. catarrhalis (MCAT), 19 Corynebacterium spp. (CSP), and 17 Bacillus

**Results**: Susceptibility (S) and activity (μg/ml) to RO based on usual cephalosporin breakpoints follow (MIC<sub>50</sub>/MIC<sub>90</sub>/%≤8 μg/ml) MSSA (0.5/0.5/100), MRSA (1/2/100), MSCoNS (0.12/0.25/100), MRCoNS (1/2/100), SPN (≤0.015/0.25/100), VgS (0.03/0.5/99) BHS (≤0.015/≤0.015/100), E. faecalis (0.5/16/89), E. faecium (>32/>32/22), CSP (0.06/>32/80), Bacillus spp. (0.12/1/100), HI (0.06/0.06/100), and MCAT (0.06/0.5/100). RO activity surpassed ceftriaxone (CTX) and cefepime (CPM) against MRSA (CPM 17%) S; CTX 2% S) and MRCoNS (CPM 63%; CTX 37%). All SPN were inhibited by RO at ≤1 μg/ml compared to CTX (74% S) and CPM (77% S). Increasing Pen-R influenced RO MICs in SPN (MIC<sub>so</sub> in μg/ml): Pen-S at ≤0.015, Pen-I at 0.12, and Pen-R at 0.25. S rate for VgS to RO (99%) was higher than CTX (80%) and CPM (79%). RO was much more potent against enterococci (62%) compared to other cephems (1-2%). HI susceptibility to RO did not decline in the \(\mathbb{G}\)-lact + strains, but BLNAR isolates (21) showed elevated MICs (MIC<sub>90</sub>, 0.25).

**Conclusions**: Overall RO showed excellent activity against the tested pathogens displaying various resistance phenotypes and should be explored for treatment of MRS and drug-resistant streptococci.

#### INTRODUCTION

Methicillin (oxacillin) resistance among staphylococci (MRS) continues to grow in occurrence world wide and this fact has contributed to escalating rates of glycopeptide use and associated resistances. Other alternative agents representing novel antimicrobial classes (streptogramins, oxazolidinones) have reached clinical use, but have generally been reserved for vancomycin-resistant strains of enterococci (VRE) or staphylococci (VISA). Due to cross-resistance, other ß-lactams [NCCLS, 2000 and 2001] are considered of limited clinical value, generally because of a shared low affinity for the resistance-associated target, PBP 2. However, several older ß-lactams (aminopenicillins, cefotaxime, cefmandole, cefmetazole) retained higher binding to PBP 2` (a transpeptidase) and other ß-lactams such as the carbapenems or penems have alternative, primary PBP target sites. These facts have fostered the continued studies of safe \(\mathbb{G}\)-lactams (L-695.256; MC-02.331; OPC-20001; SM-17466) to address emerging MRS problems

Ro63-9141 is a novel pyrrolidone 3'-ylidenemethyl cephalosporin to be used clinically as Ro65-5788, a soluble prodrug for parenteral administration (Figure 1). This compound (developed by F. Hoffmann-LaRoche Ltd and Basilea Pharmaceutica, Basel, Switzerland) has a reported broad spectrum of activity against Gram-positive and -negative organisms. Activity against MRS (MIC<sub>oo</sub>, 4 μg/ml) indicates potential clinical use, and the MIC<sub>90</sub> versus penicillin-resistant Streptococcus pneumoniae was only 2 μg/ml. Inhibition of Enterobacteriaceae by Ro63-9141 was similar to that of ceftriaxone or cefotaxime and the species most refractory to Ro63-9141 inhibition were *Pseudomonas aeruginosa*, VRE, anaerobes, and *Proteus vulgaris* and extended-spectrum ß-lactamaseproducing strains among the enteric bacilli.

This investigation was designed to confirm earlier presentations by the manufacturer about the Ro63-9141 spectrum of activity. A world wide sample of organisms was selected from recent resistance surveillance trials and tests were of reference quality versus nearly 2,000 isolates.

### MATERIALS AND METHODS

Antimicrobials tested. The Ro63-9141 (active component) was provided by F. Hoffmann-LaRoche Ltd. (Basel, Switzerland) and other comparators were purchased (Sigma Chemical, St. Louis, MO) or obtained from their manufacturers in the United States (US). A total of 23 agents were tested, only 15 are presented here (each selected by tested species).

**Organisms tested**. Over 1,700 strains were tested in this first phase study that were derived from surveillance trials world wide between 1997 and 2000. Larger numbers of resistant phenotypes were selected among the Gram-positive cocci to challenge the Ro63-9141 spectrum. The following organisms comprised the tested collection: 146 S. aureus (96 MRSA), 116 coagulase-negative staphylococci (CoNS; 90 MRCoNS), 130 Enterococcus spp. (55 VRE), 519 S. pneumoniae (258 penicillin non-susceptible), 85 viridans group streptococci (47 penicillin non-susceptible), 103 ß-haemolytic streptococci (12 macrolide-resistant), 418 H. influenzae (255 ampicillin-resistant; 21 BLNAR), 186 M. catarrhalis (167 penicillin-resistant), 19 Corynebacterium spp. and 17 Bacillus spp. All strains were identified by at least two laboratories. The strains were stored at -70∞C or below until processed.

**Susceptibility testing methods**. All tests were performed using the reference broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS) [NCCLS, 2000] in frozen-form trays produced by the investigators. The cation-adjusted Mueller-Hinton broth (CA-MHB) was modified for various organisms as follows: for *H. influenzae* the HTM formulation was used; and for the streptococci, Corynebacterium spp. and Bacillus spp. a 5% lysed horse blood CA-MHB was employed. All tests followed NCCLS technical details for incubation temperature and environment, incubation time before determining endpoints and quality control (QC). The QC organisms utilized were: E. coli ATCC 25922 and 35218, S. aureus ATCC 29213, E. faecalis ATCC 29212, H. influenzae ATCC 49247 and S. pneumoniae ATCC 49619.

The interpretive criteria were those published in the NCCLS M100-S11 [2001] and ≤ 8 µg/ml was used for Ro63-9141 versus enterococci, Enterobacteriaceae and staphylococci. Tentative Ro63-9141 breakpoints for *Haemophilus* and streptococci were set at  $\leq$  2 and  $\leq$  1  $\mu$ g/ml, respectively.

Chemical structures of Ro 63-9141 and its prodrug Ro 65-5788

#### RESULTS

• Ro63-9141 (RO) was at least eight-fold more active than ceftriaxone or cefepime against staphylococci and the MIC<sub>90</sub> versus MRSA isolates was 2 ug/ml (highest MIC, 2 ug/ml; see Table 1).

• S. aureus and CoNS were equally susceptible to RO. Vancomycin and Synercid® also exhibited spectrums ranging from 91.7 to 100.0%.

• RO was the most potent agent tested against the streptococci. S. pneumoniae RO MICs varied with the penicillin susceptibility as follows: penicillin-susceptible strains MIC<sub>20</sub> at  $\leq 0.015 \,\mu \text{g/ml}$ ; penicillin-intermediate strains MIC<sub>20</sub> at 0.12  $\mu \text{g/ml}$ ; and penicillinresistant strains MIC<sub>oo</sub> at 0.25 μg/ml (Table 2).

• viridans group streptococci also had RO MIC<sub>on</sub> results increasing with penicillin susceptibility category e.g. 0.06 to 0.025 to 1 μg/ml. All β-haemolytic streptococci were highly susceptible to RO (MIC range, ≤ 0.015 - 0.06 μg/ml). Only one viridans group streptococcal isolate had a RO MIC > 1 μg/ml.

• Enterococci were less susceptible to RO, but 88.5% of *E. faecalis* strains had MICs at ≤ 8 μg/ml; a spectrum superior to vancomycin (67.2%), but inferior to linezolid and ampicillin for this collection (Table 3).

• Only 22.0% of *E. faecium* were susceptible to RO, equal to ampicillin. Nearly one-half of these strains were vancomycin-resistant,

• All the *Bacillus* strains tested were susceptible to RO (MIC<sub>on</sub>, 1 μg/ml). *Corynebacterium* spp. were 100% susceptible to RO whereas only 22.2% strains of Corynebacterium jeikeium were inhibited by RO (Table 4).

• For *H. influenzae* there was no change in the MIC<sub>50</sub> (0.03 μg/ml) and MIC<sub>90</sub> (0.06 μg/ml) values based on β-lactamase positive phenotype. ß-lactamase-negative ampicillin-resistant (BLNAR) H. influenzae isolates (n=21) showed slightly elevated MICs (MIC<sub>50</sub>, 0.12 μg/ml, MIC<sub>oo</sub>, 0.25 μg/ml). All three fluoroquinolone-resistant strains tested were susceptible to RO (Table 5).

• M. catarrhalis isolates lacking β-lactamase were significantly more susceptible (MIC<sub>oo</sub>, 0.015 μg/ml) to RO than their β-lactamasepositive counterparts (MIC<sub>on</sub>, 0.5 μg/ml; Table 5).

## CONCLUSIONS

• Overall Ro63-9141 showed excellent in vitro activity (superior to comparison \( \mathbb{G}\)-lactams) against the Gram-positive and respiratory tract pathogens displaying various resistance phenotypes.

• Our results confirm and extend the previous findings that Ro63-9141 has a great potential as a treatment option against a variety of organisms including MRS, DRSP and some enterococci (not VRE).

• As described in earlier literature, its low affinity to altered penicillin binding proteins and poor substrate characteristics toward ß-lactamases is reflected in the low MIC values against resistant Staphylococcus and other Gram-positive and -negative (not shown here) species tested.

• Ro63-9141 shows favorable pharmacokinetic properties and low serum protein binding that warrants its exploration for treatment of infections caused by selected resistant Gram-positive pathogens.

**Table 1**. Antimicrobial activity of Ro63-9141 and comparison agents tested against *Staphylococcus* spp. MIC (μg/ml) Antimicrobial agent % susceptible (MIC)<sup>a</sup> Organism (no. tested) 100.0 Ro63-9141 oxacillin-susc. (50) Ceftriaxone 100.0 Cefepime 100.0 Synercid<sup>®</sup> 0.25-0.5 100.0 100.0 Vancomycin Ro63-9141 100.0 oxacillin-resist. (96) 0.0 >32 Ceftriaxone ≤2->16 0.0 Cefepime >16 >16 2->16 0.25->8 Synercid<sup>®</sup> 0.5 91.7 100.0 Vancomycin 0.5-4 Coag.-neg staphylococo oxacillin-susc. (26) Ro63-9141 100.0 Ceftriaxone ≤0.25-8 100.0 0.5 ≤0.12-2 100.0 Cefepime Synercid<sup>®</sup> 0.25 0.12-0.5 100.0 0.5 100.0 Vancomycin 100.0 Ro63-9141 oxacillin-resist. (90) 2->32 Ceftriaxone 0.0 Cefepime >16 0.5->16 0.0

a. MIC interpretation criteria of the NCCLS [2001] and ≤ 8 μg/ml used for Ro63-9141.

**Table 2**. Antimicrobial activity of Ro63-9141 and five other comparison drugs tested against three groups

0.25

0.12->8

0.5 -> 4

≤0.5->1

≤0.06->8

0.25 -> 4

≤0.015-0.06

91.2

61.8

7.7

7.7

88.3

99.0

100.0

94.4 98.9

% susceptible (MIC)<sup>a</sup> Antimicrobial agent S. pneumoniae Penicillin-susceptible (261) 100.0 ≤0.25 89.8 Erythromycin 0.5 ≤0.25->32 ≤0.03->4 99.2 Levofloxacin Trimethoprim/Sulfamethoxazole ≤0.5 ≤0.5->4 85.4 100.0 Penicillin-intermediate (144) Ro63-9141 ≤0.05-0.5 ≤0.008-8 97.2 Ceftriaxone ≤0.06-2 96.5 Cefepime Erythromycin ≤0.25->32 45.1 ≤0.03-1 100.0 Levofloxacin 46.5 Trimethoprim/Sulfamethoxazole ≤0.5->4 100.0 Penicillin-resistant (114) Ceftriaxone 0.5-8 61.5 77.1 ≤0.06-2 Cefepime 22.8 ≤0.25->32 Erythromycin 0.12->4 99.1 Levofloxacin Trimethoprim/Sulfamethoxazole 7.9 ≤0.5->4 viridans group 100.0 Penicillin-susceptible (38) Ro63-9141 ≤0.25 ≤0.25 Ceftriaxone ≤0.25-0.5 100.0 ≤0.12 0.5 ≤0.12-1 100.0 Cefepime 60.5 Erythromycin ≤0.06->8 0.25-2100.0 Levofloxacin 86.8 Trimethoprim/Sulfamethoxazole ≤0.5 ≤0.5->1 100.0 ≤0.015-1 Penicillin-intermediate (34) ≤0.25 ≤0.25-2 97.1 Ceftriaxone 91.2 Cefepime ≤0.12 ≤0.12-4 52.9 ≤0.06->8 Erythromycin 0.12

≤0.5

≤0.06

0.5

≤0.015

0.06

0.03-32 92.3 Penicillin-resistant (13) Ro63-9141 1->32 Ceftriaxone 0.5->16 15.4 Cefepime Erythromycin 15.4 0.12->8 0.5-2 100.0 Levofloxacin ≤0.5->1 Trimethoprim/Sulfamethoxazole ≤0.015-0.06 100.0 ß-haemolytic (103)b Ro63-9141 ≤0.25 ≤0.25 ≤0.25 100.0 Ceftriaxone ≤0.12-0.25 100.0 Cefepime ≤0.12 ≤0.12

a. MIC interpretation criteria of the NCCLS [2001] and ≤ 1 μg/ml used for Ro63-9141.

Erythromycin

Levofloxacin

Penicillin

Levofloxacin

Trimethoprim/Sulfamethoxazole

b. Includes group A (48 strains), group B (36 strains), group C (eight strains), group F (one strain), and group G (10 strains).

**Table 3**. Antimicrobial activity of Ro63-9141 and comparison agents tested against *Enterococcus* spp.<sup>a</sup>

MIC (µg/ml)

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Organism (no. tested)	Antimicrobial agent	50%	90%	Range	% susceptible (MIC) <sup>b</sup>	
E. faecalis (61)	Ro63-9141	0.5	16	0.12->32	88.5	
	Ampicillin	2	4	1->16	93.4	
	Vancomycin	2	>16	0.5->16	67.2	
	Teicoplanin	0.25	>16	≤0.12->16	83.6	
	Synercid <sup>®</sup>	8	>8	1->8	6.6	
	Linezolid	2	2	0.5-2	100.0	
	Gentamicin (HL)	≤500	>1000	≤500->1000	54.1	
E. faecium (50)	Ro63-9141	>32	>32	0.25->32	22.0	
	Ampicillin	>16	>16	≤0.12->16	24.0	
	Vancomycin	2	>16	≤0.12->16	52.0	
	Synercid <sup>®</sup>	0.5	>8	0.25->8	68.0	
	Teicoplanin	1	>16	≤0.12->16	52.0	
	Linezolid	2	2	≤0.06-2	100.0	
	Gentamicin (HL)	≤500	>1000	≤500->1000	54.0	
Other Enterococcus spp. (17)	Ro63-9141	0.5	>32	0.12->32	82.3	
	Ampicillin	1	>16	0.5->16	82.3	
	Vancomycin	4	8	0.5-16	64.7	
	Teicoplanin	0.5	1	≤0.12->16	94.1	
	Linezolid	2	2	1-2	100.0	
	Synercid <sup>®</sup>	2	8	0.25->8	11.8	
	Gentamicin (HL)	≤500	>1000	≤500->1000	82.3	

a. Approximately one-third to one-half of isolates were glycopeptide-resistant. b. MIC interpretation criteria of the NCCLS [2001] and ≤ 8 μg/ml used for Ro63-9141.

**Table 4**. Antimicrobial activity of Ro63-9141 and vancomycin tested against rare Gram-positive pathogens.

Organism (no. tested)	Antimicrobial agent	50%	MIC (μg/ml) 90%	Range	% susceptible (MIC) <sup>a</sup>
Corynebacterium jeikeium (9)	9) Ro63-9141	>32	-	0.06->32	22.2
	Vancomycin	0.5	-	0.25-1	100.0
Corynebacterium spp. (10)	Ro63-9141	0.06	>32	≤0.015->32	80.0
	Vancomycin	0.5	0.5	≤0.06-1	100.0
Bacillus spp. (17)	Ro63-9141	0.12	1	≤0.015-2	100.0
	Vancomycin	0.12	1	≤0.05-2	100.0

a. MIC interpretation criteria of the NCCLS [2001] and ≤ 8 µg/ml used for Ro63-9141.

**Table 5**. Antimicrobial activity of Ro63-9141 and four comparison drugs tested against commonly isolated Gram-negative respiratory tract pathogens.

Organism (no. tested)	Antimicrobial agent	50%	MIC (μg/ml) 90%	Range	% susceptible (MIC) <sup>b</sup>
H. influenzae (397)	Ro63-9141	0.06	0.06	≤0.015-0.25	100.0
	Ceftriaxone	≤0.008	0.03	≤0.008-0.03	100.0
	Ampicillin	≤0.015	8	≤0.5->16	64.2
	Amoxicillin/Clavulanate	0.5	2	≤0.25-8	99.2
	Ciprofloxacin	≤0.015	1	≤0.015-2	99.7
H. influenzae BLNAR (21)	Ro63-9141	0.12	0.25	0.06-0.5	100.0
M. catarrhalis (186)	Ro63-9141	0.06	0.5	≤0.015-1	100.0
	Ceftriaxone	0.12	0.5	≤0.008-1	100.0
	Penicillin	4	>4	≤0.03->4	10.2
	Amoxicillin/Clavulanate	≤0.25	≤0.25	≤0.25-0.5	100.0
	Ciprofloxacin	0.03	0.06	≤0.015-0.06	100.0

a. MIC interpretation criteria of the NCCLS [2001] and  $\leq$  2  $\mu$ g/ml used for Ro63-9141.

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