

## Abstract

**Objectives:** To determine the mechanisms of ketolide resistance in *H. influenzae*. Ketolide resistance is very rare in *H. influenzae* and is usually associated with a variety of ribosomal mutations. We report on the ribosomal mutations detected in 9 telithromycin (TELI)-resistant *H. influenzae* isolates found in the SENTRY Program (2009) and assess the activity of solithromycin (SOLI, formerly CEM-101), a new fluoroketolide in clinical development.

**Methods:** 1,198 *H. influenzae* isolates obtained from patients with community-acquired bacterial pneumonia in 24 countries were tested for susceptibility to TELI by CLSI methods (M07-A8 and M100-S21) as part of the SENTRY Antimicrobial Surveillance Program during 2009. Only nine (0.8%) isolates were found to be TELI-resistant (MIC,  $\geq 16$  mg/L). Extended MICs were performed by Etest and strains were screened for mutations in the 23S rRNA, L22 and L4 proteins by PCR and DNA sequencing.

**Results:** Seven different mutation patterns were observed in 8 of the 9 strains. No mutations were detected in the genes sequenced for one strain (Isolate 3042). The highest TELI MIC values ( $>256$  mg/L) were found in two geographically diverse (Sweden and USA) strains with a 23S rRNA A2059G mutation. *H. influenzae* with L4 and L22 riboprotein mutations showed TELI MIC values from 32 to 256 mg/L. TELI was 1.5- to at least 4-fold more active than azithromycin (AZI); and SOLI was 2- to at least 4-fold more active than TELI. Against all 1,198 *H. influenzae* isolates, the MIC<sub>50/90</sub> for TELI and SOLI were 1/4 and 1/2 mg/L, respectively.

**Conclusions:** Ketolide resistance in *H. influenzae* continues to be rare ( $<1\%$ ) globally. Resistance was found to be associated with a variety of ribosomal mutations and was widely distributed geographically thus suggesting a lack of both local and global spread. Although solithromycin MIC results were 2- to at least 4-fold lower than telithromycin against these strains, cross-resistance to solithromycin was confirmed. The mechanism of ketolide resistance in one UK strain is under further investigation.

## Introduction

Macrolide resistance in *Haemophilus influenzae* is complex. Almost all *H. influenzae* possess an intrinsic efflux pump homologous to the *acrAB* efflux mechanism of *Escherichia coli* that lowers the activity of macrolide, lincosamide, streptogramin B and ketolide antimicrobial agents compared to strains that do not have this mechanism. For azithromycin, higher MIC values (and hence resistance), although rare, have resulted from mutations in the riboproteins L4 and L22 and/or 23S rRNA. Recently, macrolide resistance caused by acquired 23S rRNA methylase genes (*erm*[A], *erm*[B], *erm*[C], *erm*[F]) and/or macrolide efflux (*mef*[A]) have been described.

Telithromycin is a ketolide antimicrobial agent and is a semi-synthetic derivative of erythromycin A. Ketolides bind to 23S at domain II, as well as domain V, and retain good activity against macrolide-resistant strains, hence telithromycin resistance is rare. Ketolide resistance in *H. influenzae* is associated with mutations in L4, L22 and 23S rRNA.

Solithromycin (CEM-101) is the first fluoroketolide selected as a candidate for oral and/or parenteral therapy of community-acquired bacterial pneumonia (CABP) and other infections, such as urethritis and *Mycobacterium avium* infections. Solithromycin has demonstrated excellent activity (MIC<sub>50/90</sub>, 1/2 mg/L) against European *H. influenzae* isolates and is typically two- to eight-fold more active than telithromycin.

In this study, we investigate the mechanisms leading to elevated ketolide (telithromycin) MIC values found in nine *H. influenzae* strains isolated during the global SENTRY Antimicrobial Surveillance Program (2009) and describe the activity of solithromycin against these strains.

## Materials and Methods

**Bacterial strain collection.** During 2009, a total of 1,198 *H. influenzae* isolates were obtained from patients with community-acquired bacterial pneumonia in 24 countries, and were tested for susceptibility to telithromycin by CLSI methods (M07-A8 and M100-S21) as part of the SENTRY Program. Species identifications were performed by the submitting laboratories with confirmation by the central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA).

**Susceptibility test methods.** All isolates were tested for susceptibility to telithromycin, solithromycin and comparators by reference broth microdilution methods using the CLSI recommendations (M07-A8, 2009). Susceptibility testing was performed by using validated broth microdilution panels manufactured by TREK Diagnostics Systems (Cleveland, Ohio, USA). Further validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S21, 2011) quality control (QC) strains, including *H. influenzae* ATCC 49247. Categorical interpretation of comparator MIC values was performed according to CLSI (M100-S21, 2011) criteria, when available. Extended MIC results for azithromycin were performed by Etest (bioMérieux).

**Molecular test methods.** Telithromycin-resistant strains (MIC  $\geq 4$  mg/L) were screened for *erm*(A), *erm*(B), *erm*(C), *erm*(F) and *mef*(A/E) resistance genes by PCR, and for mutations in the 23S rRNA, L22 and L4 proteins by PCR and DNA sequencing.

## Results

- In 2009, tested against all 1,198 *H. influenzae* isolates, solithromycin (MIC<sub>90</sub>, 2 mg/L) and azithromycin (MIC<sub>90</sub>, 2 mg/L) were two-fold more active than telithromycin (MIC<sub>90</sub>, 4 mg/L; Table 1). Solithromycin demonstrated greater potency than both azithromycin and telithromycin for many strains with the percentage inhibited at a MIC of  $\leq 1$  mg/L being 81.1, 66.2 and 52.8%, respectively (Table 1).
- Only nine strains (0.8%) were found to be resistant to telithromycin by CLSI interpretive criteria ( $\geq 16$  mg/L). Although the solithromycin MIC value for each strain was two- to four-fold lower than both telithromycin and azithromycin, the solithromycin MIC range of 8-128 mg/L indicates that solithromycin is also adversely affected by these mechanisms (Table 2). These nine strains were geographically diverse.
- The A2059G mutation in the peptidyl transferase center of 23S rRNA (2 strains) was associated with the highest telithromycin MIC values ( $>256$  mg/L). The various amino acid changes found in the L4 and L22 riboproteins were also associated with high telithromycin MIC results (32-256 mg/L; Table 2). The mechanism of ketolide resistance in one United Kingdom strain is under further investigation.

Table 2. Summary of phenotypic and genotypic characteristics of telithromycin-resistant *H. influenzae*

Isolate	Country	Mutation	MIC in mg/L		
			TELI <sup>a</sup>	SOLI <sup>b</sup>	AZI <sup>c</sup>
279	Sweden	23S_A2059G	>256	64	>256
1540	USA	23S_A2059G	>256	128	>256
3904	Hong Kong	L22_87insRVMPRVMP88	256	128	>256
822	USA	L22_del95RIL	32	16	128
3302	USA	L22_G91D	32	16	48
684	Chile	L22_R88P, L22_R99L	128	64	>256
995	USA	L4_63insKG64	32	8	48
3432	Australia	L4_T64K	64	16	>256
3042	UK	None found	256	128	>256

a. TELI = telithromycin.  
b. SOLI = solithromycin.  
c. AZI = azithromycin

Table 1. MIC frequency and cumulative percent inhibited distribution of solithromycin, telithromycin and azithromycin against 1,198 *H. influenzae* isolated globally in 2009.

Antimicrobial	MIC (mg/L)										MIC <sub>50</sub>	MIC <sub>90</sub>
	$\leq 0.06$	0.12	0.25	0.5	1	2	4	8	16	>16		
Telithromycin	1 (0.1)	4 (0.4)	8 (1.1)	77 (7.5)	542 (52.8)	436 (89.2)	115 (98.8)	6 (99.3)	0 (99.3)	9 (100.0)	1	4
Solithromycin	3 (0.5)	7 (0.8)	22 (2.7)	284 (26.4)	655 (81.1)	204 (98.1)	13 (99.2)	1 (99.3)	3 (99.5)	6 (100)	1	2
Azithromycin	- <sup>a</sup>	-	-	170 (14.2)	623 (66.2)	335 (94.2)	60 (99.2)	1 (99.3)	0 (99.3)	9 (100)	1	2

a. - = untested concentration.

## Conclusions

- Ketolide resistance in *H. influenzae* continues to be rare ( $<1\%$ ) globally. Resistance was found to be associated with a variety of ribosomal mutations and was widely distributed geographically, thus suggesting a lack of both local and global spread.
- Although solithromycin MIC results were two- to at least four-fold lower than telithromycin and azithromycin against these strains, cross-resistance to solithromycin appears to be confirmed.

## Selected References

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