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Macrolide-Induction, Inoculum Effect and Kill-Curve Analysis of Telithromycin Tested Against *S. pneumoniae* Strains with Characterized MLS_B Resistances

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AMENDED ABSTRACT

Background: Telithromycin (TEL) is a semisynthetic derivative of the macrolide class that has activity advantage over MLS_B agents against *S. pneumoniae* (SPN). This study evaluates erythromycin (ERY) induction of resistance ($R_r \geq 4$ µg/ml) to TEL, inoculum concentration (conc) effects elevating TEL MICs and kill-curve kinetics of TEL against SPN isolates with known MLS_B-R mechanisms.

Methods: 51 SPN isolates with MLS_B-R were tested including: 10 strains each with M or MLS(B) phenotypes; 10 strains with confirmed *erm*(B) or *mef*(A); 5 strains with *erm*(B) and *mef*(A); and 6 strains with target-site modification in the 23S rRNA and/or L22 riboprotein mutations. MLS_B and TEL MICs were determined using NCCLS broth microdilution methods. Characterized R mechanisms were detected using multiplex rapid cycle PCR methods. Induction experiments used 0.12 µg/ml of ERY + TEL, inoculum concs included 10^3, 10^5 and 10^7 CFU/ml and kill-curve analysis was performed using fixed 1, 2 and 4 µg/ml TEL concentrations (10 strains): TEL-S breakpoint [BP] was at ≤ 1 µg/ml.

Results: Baseline TEL MICs ranged from ≤ 0.06 - 2 µg/ml and 20 strains increased MIC $\geq$ 2-fold (14), $\geq$ 4-fold (5) and $\geq$ 8-fold (1) in the presence of ERY distributed among all MLS_B-R groups (8 strains reached BP). The inoculum concentration influenced the TEL MICs with 23 strains having lower MICs (usually 2-fold) at 10^3 versus 10^5 and higher (23 strains; 2 to 16-fold) at 10^7 (4 strains moved into the R category). Most *erm*(B)± *mef*(A) strains tested by kill-curve had stable bacterial counts at T_0-T_8 and regrowth (T_8-T_{24}) at all concentrations. The *mef*(A) strains had significant reduction in growth at 2 and 4 µg/ml and generally poor activity was noted at 1 µg/ml. All concentrations showed significant kill against strains negative for *erm*(B) or *mef*(A) mediated resistances.

Conclusions: SPN strains with MLS_B-R mechanisms can have TEL MICs influenced by inoculum concentration and ERY induction. Kill curve analysis showed significant decreases in bacterial counts (T_{24}) only with TEL 2 and 4 µg/ml concentrations.

INTRODUCTION

The ketolide class of antimicrobial agents offers an advantage over other macrolide-lincosamide-streptogramin (MLS_B) compounds against *Streptococcus pneumoniae* due to the lack of cross resistance. Telithromycin is a ketolide that has been released for clinical use against community-acquired respiratory infections including pneumonia, sinusitis and acute exacerbations of chronic bronchitis. *S. pneumoniae* is a primary pathogen related to these indications and resistance to penicillin and MLS_B agents has been documented at alarming rates in some countries. Although penicillin resistance rates vary greatly from region to region, overall resistance rates are approximately 40% worldwide. Macrolide-resistant *S. pneumoniae* strains are also commonly isolated with resistance mediated by efflux and methylase mechanisms.

Cross resistance to MLS_B agents can be expressed constitutively or inducibly, and erythromycin has been shown to strongly induce resistance to other MLS_B agents. In contrast, ketolides have not been shown to be inducibly resistant and retain activity against MLS_B-resistant *S. pneumoniae* regardless of the mechanism of resistance. Telithromycin, among the ketolides, has been shown to have little resistance inducing potential to other members of the MLS_B class. The purpose of this study was to evaluate the ability of erythromycin to induce telithromycin resistance among *S. pneumoniae* having well characterized MLS_B resistance mechanisms. We also evaluated the kill-curve kinetics and the effect of inoculum concentration on the telithromycin MIC values tested against these isolates.

MATERIALS AND METHODS

Organisms tested. The SENTRY Antimicrobial Surveillance Program collected unique erythromycin-resistant *S. pneumoniae* isolates from patients with community-acquired pneumonia during 2001 - 2003. A subset of 51 strains, the majority of which were genotypically characterized for the presence of resistance mechanisms, were used in this study. Isolates were phenotypically and/or genotypically methylase (20 strains)- or efflux (20 strains) resistant-positive. Additionally, five strains were confirmed to have the presence of both resistance mechanisms and six strains were negative for either *mef*(A) or *erm*(B). The six negative isolates had mutations in the 23S ribosomal target and/or L22 protein region.

Susceptibility test methods. Isolates were tested for susceptibility to MLS_B agents using NCCLS methods and commercially prepared dry-form broth microdilution panels (TREK Diagnostics, Cleveland, OH). Resistance induction was studied using 0.12 µg/ml of erythromycin/telithromycin containing wells and examining deviations of the MIC versus telithromycin tested alone. Inoculum densities of 10^3, 10^5 and 10^7 CFU/ml were used to determine the effect of microbial concentration on telithromycin MIC values.

Molecular analysis. Multiplex rapid-cycle PCR screening and probe detection for *erm*(B) and *mef*(A) was performed on select strains. Strains negative for these genes were rRNA sequenced.

RESULTS

- Telithromycin MIC values ranged from ≤ 0.06 - 2 µg/ml for the MLS_B resistant strains used for these studies (data not shown).
- In the presence of erythromycin (induction), telithromycin MIC values increased for 40% (*erm*[B]), 45% (*mef*[A]), 20% (*erm*[B] + *mef*[A]) and 17% (*erm*[B] and *mef*[A] negative) of the strains with these characterized resistance patterns (Table 1).
- The effect of inoculum concentration on telithromycin is shown in Table 1. At lower inoculum concentrations the percentage of strains with lower MIC values was 55%, 35%, 100% and 17% for *erm*(B), *mef*(A), *erm*(B) + *mef*(A) and negative for *erm*(B) and *mef*(A), respectively. Higher MIC values were noted at higher inoculum concentrations for 45%, 50%, 60% and 17% of strains with these resistance mechanisms, respectively.
- Time kill studies (Table 2) indicate that strains with an *erm*(B) resistance mechanism were typically held static by telithromycin from T_0 to T_8 hours with regrowth after 8 hours of incubation regardless of the ketolide concentration tested (Figure 1). Strains with other MLS_B resistance mechanisms were also held static at a telithromycin concentration of 1 µg/ml with cidal activity identified only at higher concentrations (Figure 2 and 3).

Table 1. Macrolide induction (+ erythromycin) and inoculum effect when testing telithromycin against *S. pneumoniae* with characterized MLS_B resistance.

		MIC variation (log_2 dilutions)									
Resistance mechanism	Test condition	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
erm(B) (20)	+ erythromycin[a]	-	-	-	-	12	6	1	1	-	-
	low inoculum[b]	-	1	3	7	9	-	-	-	-	-
	high inoculum[c]	-	-	-	-	11	3	2	1	3	-
mef(A) (20)	+ erythromycin	-	-	-	-	11	6	3	-	-	-
	low inoculum	-	-	-	7	13	-	-	-	-	-
	high inoculum	-	-	1	-	9	9	1	-	-	-
erm(B) + *mef*(A) (5)	+ erythromycin	-	-	-	-	4	1	-	-	-	-
	low inoculum	-	-	1	4	-	-	-	-	-	-
	high inoculum	-	-	-	1	1	2	1	-	-	-
negative (6)[d]	+ erythromycin	-	-	-	-	5	1	-	-	-	-
	low inoculum	-	-	-	1	5	-	-	-	-	-
	high inoculum	-	-	-	-	5	1	-	-	-	-

a. An erythromycin concentration of 0.12 µg/ml was used in the induction experiment.
b. Low inoculum concentration of 10^3 CFU/ml was compared to standard inoculum (10^5 CFU/ml).
c. High inoculum concentration of 10^7 CFU/ml was compared to standard inoculum (10^5 CFU/ml).
d. Strains had 23S rRNA target site modifications and/or L22 riboprotein mutations.

Table 2. Kill curve results for telithromycin tested at three concentrations (1, 2 and 4 µg/ml) against *S. pneumoniae* with MLS_B resistance including four *erm*(B), four *mef*(A), one *erm*(B) and *mef*(A) and one strain with 23S and L22 mutations.

		Concentration (log_{10} CFU/ml) at T				
Organism (telithromycin MIC, µg/ml)	Test concentration (µg/ml)	0	4	8	24	Activity category[a]
S. pneumoniae						
erm(B)						
62-3234B (1)	1	7.2E5	1.1E6	9.1E5	3.8E8	Static[b]
	2	-	7.9E5	2.2E5	5.5E8	Static[b]
	4	-	6.5E5	5.8E5	2.0E8	Static[b]
58-4110B (1)	1	9.0E5	1.5E6	1.6E6	3.8E8	Static[b]
	2	-	1.0E6	8.8E5	4.4E8	Static[b]
	4	-	6.8E5	3.6E5	5.9E7	Static[b]
301-1368B (1)	1	5.5E5	1.3E6	2.7E6	4.7E8	Static[b]
	2	-	1.3E6	9.2E5	3.3E8	Static[b]
	4	-	7.3E5	7.4E5	4.4E7	Static[b]
81-3561B (0.5)	1	5.0E5	2.9E5	1.7E4	3.1E6	Static[b]
	2	-	2.1E5	9.2E4	5.8E5	Static
	4	-	1.6E5	5.7E4	2.0E3	Static
erm(B) + *mef*(A)						
27-1371B (0.5)	1	7.0E5	1.0E5	4.8E4	1.5E3	Static
	2	-	8.6E4	4.3E4	6.0E2	Cidal
	4	-	8.0E4	2.0E4	2.9E2	Cidal
mef(A)						
30-2917B (0.5)	1	4.3E5	6.8E4	3.0E3	4.4E7	Static[b]
	2	-	6.8E4	2.5E4	1.5E2	Cidal
	4	-	7.9E4	1.7E4	1.3E2	Cidal
29-2185B (0.5)	1	8.2E5	4.9E5	1.9E5	1.5E6	Static
	2	-	3.2E5	2.2E5	1.5E3	Static
	4	-	3.2E5	1.8E5	7.7E2	Cidal
101-6109B (0.5)	1	5.0E5	3.2E4	9.1E3	4.8E2	Cidal
	2	-	2.1E4	5.0E3	1.4E2	Cidal
	4	-	2.0E4	4.2E3	6.0E1	Cidal
29-2182B (0.5)	1	9.9E5	6.1E4	1.5E3	6.0E3	Static
	2	-	4.9E4	1.6E3	1.0E1	Cidal
	4	-	4.4E4	6.4E2	1.0E1	Cidal
23S + L22[b]						
17-3167B (0.25)	1	6.4E5	8.5E4	7.6E3	1.3E3	Static
	2	-	1.6E4	2.2E2	3.0E2	Cidal
	4	-	8.8E3	1.1E2	1.0E1	Cidal

a. Bactericidal (cidal) activity was defined as $\geq$ three log_{10} reduction in the initial inoculum within 24 hours of incubation.
b. Indicates regrowth after eight hours.
c. Strain had a documented gene insertion (5AA) in L22 and a 23S mutation (A2059G).

Figure 1. Time kill-curve for telithromycin tested against an *erm*(B) positive strain of *S. pneumoniae*.

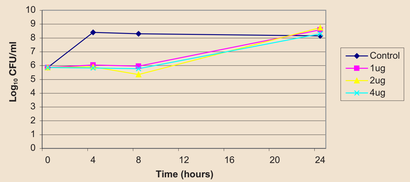


Figure 2. Time kill-curve for telithromycin tested against a *mef*(A) positive strain of *S. pneumoniae*.

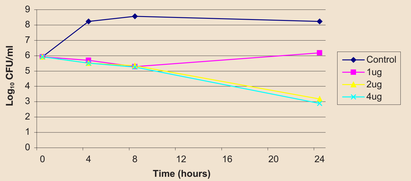
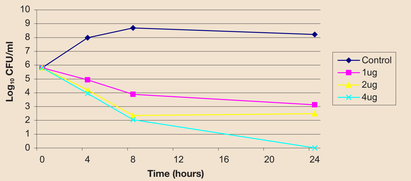


Figure 3. Time kill-curve for telithromycin tested against a *S. pneumoniae* strain with a gene insertion (5AA) in L22 and a 23S mutation (A2059G).



CONCLUSIONS

- Following erythromycin induction, telithromycin MIC values increased for *S. pneumoniae* isolates having documented MLS_B resistance mechanisms.
- A correlation between elevated telithromycin MIC values and the adverse effect of inoculum concentrations was clearly established with *S. pneumoniae*.
- Telithromycin generally produced bacteriostatic activity against *S. pneumoniae* isolates with *erm*(B) resistance mechanisms which typically showed rapid regrowth after eight hours of incubation. Strains with other MLS_B resistance mechanisms were inhibited at a bactericidal level for telithromycin concentrations of ≥ 2 µg/ml.
- The presence of MLS_B mechanisms in *S. pneumoniae* can adversely affect telithromycin MIC values when tested at higher bacterial concentrations and after erythromycin induction.