Accuracy in determining telithromycin MIC values against *Streptococcus pneumoniae*: *Haemophilus influenzae* and *Streptococcus pyogenes*: Etest vs. Broth Microdilution

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Abstract

Objectives: *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* are common causes of outpatient respiratory tract infections routinely treated with oral agents including macrolides and the new ketolide telithromycin. Macrolide resistant *S. pneumoniae* are increasingly common while telithromycin resistance is exceptionally rare in *S. pneumoniae*. Accurate susceptibility testing is essential to document *S. pneumoniae* telithromycin activity. This study documents the effects of CO_2 on telithromycin MICs when determined by Etest methodology. **Methods:** A total of 188 *S. pneumoniae*, 44 *S. pyogenes* and 83 *H. influenzae* of various phenotypes were selected from recent international surveillance trials. All Etest values were determined after incubation in 5-7% CO_2, while broth microdilution panels were tested under ambient air conditions following NCCLS guidelines. Both broth microdilution panels and Etest plates were inoculated using the same broth inoculum. Random colony counts confirmed inocula. **Results:** Telithromycin Etest and broth microdilution geometric mean (GM) MICs and fold GM increases for All isolates, macrolide sensitive and macrolide non-susceptible *S. pneumoniae* were as follows: All isolates [0.108/0.037, 2.9], macrolide sensitive [0.022/0.006, 3.7] and macrolide resistant [0.228/0.079, 2.9]. Telithromycin Etest MIC_{90} for *S. pneumoniae* increases 2-4 fold compared to broth microdilution MIC_{90} for *S. pneumoniae*. However, no susceptible to resistant categorical interpretative changes were documented. **Conclusions:** Etest is a valuable diagnostic tool, however, when testing *S. pneumoniae* against telithromycin in a CO_2 environment, MICs increase. The data suggests caution while interpreting telithromycin MICs when using telithromycin Etest strips in a CO_2 environment.

Introduction

The importance of the laboratory's *in vitro* evaluation of respiratory pathogens such as *Streptococcus pneumoniae, Haemophilus influenzae* and *Streptococcus pyogenes* has gained in acceptance due to the increased number of organisms resistant to common therapeutic agents such as penicillins and macrolides. It is important to the clinician that susceptibility information for these antimicrobials and others against pathogenic organisms be accurate and reliable.

Many laboratories worldwide have adopted the NCCLS standards and guidelines for the testing of various pathogens (1,3). For many respiratory pathogens, these procedures include the incubation of disk diffusion agar plates in a 5-7% CO_2 environment. For most organism and antimicrobial combinations this is not a problem and the values obtained from disk diffusion or Etest methodology (CO_2) correlate very well to the broth microdilution reference method (incubated in ambient air). However, in some incidences, especially with macrolides (2,5) and some quinolones (4) it has been documented that incubation in a CO_2 environment has resulted in artificially increased MICs for these compounds.

Telithromycin, the first marketed ketolide, has been tested extensively *in vitro* and quality control values and breakpoints have been established for MICs and disk diffusion. However, NCCLS quality control ranges and breakpoints established for MIC values were not determined or evaluated when CO_2 incubation was employed. This study was undertaken to determine if MIC values obtained under CO_2 incubation are comparable to those recorded for broth microdilution panels incubated under ambient air conditions.

Materials and Methods

- Isolates used in this study were collected from sites in the United States, Canada and Europe between January 2000 and December 2002.
- The phenotypes of all isolates were confirmed prior to study initiation using GLP, NCCLS guidelines and recommendations from Essential Procedures for Clinical Microbiology, American Society for Microbiology, 1998.
- A total of 315 *S. pneumoniae*, *S. pyogenes* and *H. influenzae* isolates were evaluated.
- All isolates were tested using broth microdilution reference panels under ambient air conditions, by disk diffusion and Etest with the latter two methodologies evaluated under CO_2 conditions.
- Azithromycin was included as a comparator for all three methods.
- A common inoculum for each organism was used in the preparation of the inocula for each of the three testing methodologies.
- All testing and interpretation was performed using NCCLS guidelines and standards or by recommendations of the manufacturer.
- Random colony counts were conducted on the inocula throughout the study.
- Panels and comparative agents were furnished by Trek Diagnostic (West Sussex, UK); CMI (Tualatin, Oregon). Etest was furnished by ABBIODISK (Solna, Sweden). Disks were furnished by Becton Dickinson (Sparks, Maryland)

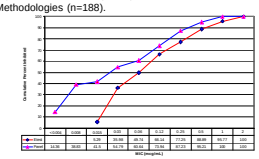
Results

Table 1: Different types and Numbers of Pheonotypic Organisms* evaluated in This Study.

Organism	Phenotype	Number
S.pneumoniae		188
	Penicillin Susceptible	50
	Penicillin Intermediate	59
	Penicillin Resistant	79
	Macrolide Resistant	44
	Fluoroquinolone Resistant	41
S.pyogenes		44
H. influenzae		83
	Beta lactamase positive	42
	Beta lactamase negative	41

*Organisms were selected for one phenotypic characteristic, however, some organisms may express more than one phenotypic characteristic.

Graph 1: Cumulative Percent Inhibited of Telithromycin Against *S.pneumoniae* Comparing Broth Microdilution and Etest Methodologies (n=188).



Graph 2: Cumulative Percent Inhibited of Telithromycin against *S.pyogenes* Comparing Broth Microdilution and Etest Methodologies (n=44).

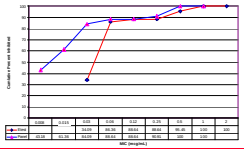


Table 2: Activity of Telithromycin and Azithromycin Against Various Phenotypes of *S. pneumoniae* When Tested Using a Broth Microdilution Methodology in Ambient Air.

		Telithromycin[1]					Azithromycin				
					(mcg/mL)					(mcg/mL)	
Phenotype[2]	N	S	I	R	MIC_{50}	MIC_{90}	S	I	R	MIC_{50}	MIC_{90}
Combined[3]	188	96	-	-	0.03	0.5	37.2	-	62.8	4	>64
PSSP[4]	50	100	-	-	0.015	0.03	100	-	-	0.12	0.12
PISP	59	100	-	-	0.03	0.25	36.6	-	63.4	4	16
PRSP	79	100	-	-	0.12	0.5	21.9	-	78.1	8	8
MacR[5]	88	100	-	-	0.12	0.5	-	-	100	>8	64
FluoroR[6]	43	100	-	-	0.03	0.5	43.9	-	56.1	4	8

[1]Telithromycin breakpoints (mcg/ml) for S. pneumoniae: Sus.=≤1; Int.=2; Res.=≥4.
[2]A total of 188 *S.pneumoniae* were tested however some organisms had multiple phenotypic characteristics.
[3]Non-random sampling. Isolates tested were biased toward resistant phenotypes
[4]Penicillin breakpoints (mcg/mL): Sus=≤0.06; Int.=0.12-1; Res.=≥2
[5]Macrolide breakpoints (mcg/mL) based on azithromycin: Sus=≤0.5; Int.=1; Res.=≥2
[6]Fluoroquinolone breakpoints (mcg/mL) based on levofloxacin: Sus=≤2; Int.=4; Res.=≥8

Graph 3: Cumulative Percent Inhibited of Telithromycin against *H.influenzae* Comparing Broth Microdilution and Etest Methodologies (n=83).

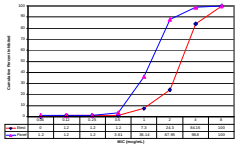


Table 3: Activity of Telithromycin Against Various Phenotypes of *S. pneumonia, S. pyogenes and H. influenzae* Comparing All Three Test Methods.[1]

	Broth Microdilution							Etest					Disk[5]
Organism	N	S	I	R	Mode	MIC_{90}	Gmean[2]	S	I	R	Mode	MIC_{90}	Gmean	S
S.pneumoniae														
Combined[4]	188	100	0	0	0.02	0.5	0.049	95.8	4.2	0	0.03	1	0.097	100
PSSP	50	100	0	0	0.02	0.03	0.014	100	0	0	0.03	0.06	0.032	100
PISP	59	100	0	0	0.02	0.25	0.049	97.6	3.4	0	0.03	0.5	0.087	100
PRSP	79	100	0	0	0.12	1	0.088	87.5	12.7	0	0.12	2	0.155	100
MacS	70	100	0	0	0.02	0.03	0.028	100	0	0	0.02	0.06	0.013	100
MacR	118	100	0	0	0.25	0.5	0.089	93.2	6.8	0	0.5	1	0.228	100
FluoroR	43	100	0	0	0	0.5	0.039	100	0	0	0.03	0.5	0.069	100
S. pyogenes [3]	44	100	0	0	0.02	0.25	0.025	100	0	0	0.06	0.5	0.058	100
H. influenzae [4]	83	98.8	1.2	0	2	4	1.636	84.1	15.9	0	4	8	2.931	100
β-Lactamase+	42	97.6	2.4	0	2	4	1.639	88.1	11.9	0	4	8	2.799	100
β-lactamase-	41	100	0	0	2	2	1.633	80.5	19.5	0	4	8	3.077	100

[1]Etest values rounded up to next 2-fold dilution value.
[2]Gmean = Geometric mean.
[3]S. pyogenes breakpoints (mcg/ml) used for telithromycin: Sus.=1; Int.=2; Res.=4
[4]*H.influenzae* breakpoints (mcg/ml) used for telithromycin: Sus.=4; Int.=8; Res.=16
[5]Disks breakpoints (mm) for S.pneumoniae and S. pyogenes: Sus.=≥19; Int.=16-18; Res.=≤15; for influenzae: Sus.=≥15; Int.=12-14; Res.=≤11
[6]A total of 188 *S.pneumoniae* were tested however some organisms had multiple phenotypic characteristics.

Conclusions

The activity of telithromycin against selected phenotypes of *S. pneumoniae*, *S. pyogenes* and *H. influenzae* in this study correlate to those found in the published literature when broth microdilution or disk diffusion is the *in vitro* testing method. However, when MICs are determined by Etest in which agar plates are incubated in CO_2 values are less reliable and must be closely monitored. As seen in the cumulative percent-inhibited graphs, higher MICs result with Etest in CO_2 and these values are not reproducible with broth microdilution tested in ambient air. Of concern is the more than 50% increase in the geometric mean for *S. pneumoniae*, *S. pyogenes* and *H. influenzae*. This could be very important as MICs for some organisms approach the breakpoint of telithromycin, a shift of 1 to 2 dilutions would make these organisms appear non-susceptible. Additional studies should be conducted to evaluate this phenomenon and determine appropriate break points for Etest containing telithromycin when incubated in a CO_2 environment. In the interim, laboratories should confirm any organisms expressing unusually high telithromycin Etest MICs or non-susceptibility by retesting using NCCLS broth microdilution or disk diffusion methodology.

References

1. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Villanova, PA, USA: NCCLS, 2003: approved standard M7-A6.
2. Gerads SH, Citron DM, Clarros MC, Goldstein EJC. Comparison of Etest to broth microdilution method for testing Streptococcus pneumoniae susceptibility to levofloxacin and three macrolides. Antimicrob Agents Chemother. 1996;40:2413-2415.
3. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Villanova, PA, USA: NCCLS, 2003: approved standard M100-S13.
4. DJ Hoban, SK Bouchillon, BM Johnson, TM Stevens, ME McCarthy, CE Gaylord, M Bailey-Person and L Eng-Wong. Accuracy in Determining MIC Values of Newer Fluoroquinolones (FQ) Against *Streptococcus pneumoniae* (SP) using Etest in a CO_2 Environment. Poster 42nd ICAAC, 2002.
5. Johnson J, Bouchillon S, Pontani D. The effect of carbon dioxide on susceptibility testing of azithromycin, clarithromycin and roxithromycin against clinical isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes* by broth microdilution and the Etest: Artemis Project-first-phase study. Clin Microbiol Infect. 1999 Jun;5(6):327-330.

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