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THE EFFECTS OF CO_2 ON THE IN VITRO ACTIVITY OF TELITHROMYCIN WHEN USING ETEST® COMPARED WITH BROTH MICRODILUTION IN 7 REFERENCE LABORATORIES

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REVISED ABSTRACT

Background: Telithromycin has been tested extensively in vitro, and the National Committee for Clinical Laboratory Standards (NCCLS) quality control ranges and breakpoints have been established for the minimum inhibitory concentration (MIC) values and disc diffusion. However, MICs were not determined or evaluated in the presence of CO_2. This study was undertaken to determine if MIC values for telithromycin obtained under CO_2 incubation were comparable to those recorded for broth microdilution panels incubated under ambient conditions.

Methods: Telithromycin MICs from 400 clinical isolates of *Streptococcus pneumoniae* (n=200), *Haemophilus influenzae* (n=100), and *Streptococcus pyogenes* (n=100) were tested in clinical laboratories using Etest® (AB Biodisk, Solna, Sweden) in CO_2 and were compared with broth microdilution MICs according to NCCLS guidelines.

Results: Telithromycin Etest MICs in CO_2 were consistently 1 $\log_2$ dilution higher than MICs of broth microdilution for *H influenzae* and 2 $\log_2$ dilutions higher for *S pneumoniae* and *S pyogenes*.

Conclusions: Due to the impact of CO_2 on telithromycin, Etest is not recommended for the routine in vitro testing of this ketolide. Should Etest be used, the appropriate $\log_2$ correction factor should be employed before reporting Etest MICs or interpretive susceptibilities.

INTRODUCTION

- Telithromycin, the first in a new class of antibiotics called ketolides, has a spectrum of activity against many gram-positive and gram-negative organisms typical of upper and lower respiratory tract infections, including resistant strains.
- Telithromycin has been tested extensively in vitro. Quality control (QC) values and breakpoints have been established for minimum inhibitory concentration (MICs) and disc diffusion zone size according to standardized microbiologic procedures set forth in protocols established by the Food and Drug Administration and the National Committee for Clinical Laboratory Standards (NCCLS).[1,2]
  - As per these protocols, MIC was determined in a broth microdilution panel incubated in ambient air.
- Etest® (AB Biodisk, Solna, Sweden) is a product that is used to determine the MIC of specific antimicrobial-bacterium combinations under defined conditions. Etest strips, impregnated with a continuous concentration gradient of the antimicrobial agent of interest, are placed in an agar plate with the bacterium isolate and the MIC value is read where the inhibition eclipse meets the antibiotic-impregnated strip.[3]
  - Fastidious organisms, such as those involved in lower respiratory tract infections, require incubation in CO_2 for this method.
- Because the NCCLS QC ranges and breakpoints for telithromycin were not determined using CO_2 incubation, errors in reporting MICs may occur when testing telithromycin with some organisms in the presence of CO_2.
- Testing pH-sensitive antimicrobial agents in a CO_2 environment may affect the resultant MIC values. This has been demonstrated with some macrolides[4-6] and quinolones,[7] as well as telithromycin.[8]
- However, there are only a few published reports of data concerning this issue in reference to telithromycin and fastidious isolates when the Etest method is used.
- Therefore, this study was undertaken to determine the quantitative difference of telithromycin MIC values obtained using Etest in a CO_2 environment compared with those recorded for broth microdilution panels incubated under ambient air conditions. The study was designed to follow an NCCLS M23 format to help eliminate biases that may exist between laboratories.

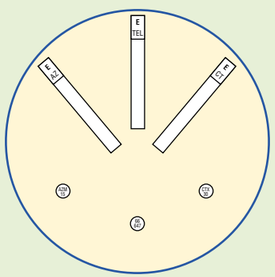
METHODS

- Isolates consisted of 400 banked, clinical isolates, less than 18 months of age, supplied by Laboratories International for Microbiology Studies (Laboratories International for Microbiology Studies, Schaumburg, Ill).
- The study group of organisms consisted of 100 strains of *Streptococcus pneumoniae*, 50 strains of *Haemophilus influenzae*, and 50 strains of *Streptococcus pyogenes*; each strain was tested twice by each laboratory.
  - These organisms were chosen because they are the pathogens most commonly seen in respiratory tract infections.
- Azithromycin was used as a positive control.[4,9]
- Seven independent reference laboratories tested each of the 200 strains twice, once by broth microdilution and once using both Etest strips and disc diffusion, over a period of 10 days in a predetermined order according to a standardized protocol.
- Each laboratory used identical lot numbers of MIC panels, Etest strips, discs, and reagents.
- All laboratories followed guidelines for testing of fastidious organisms in a CO_2 environment.

Antimicrobial susceptibility testing

- Susceptibility testing was performed using broth microdilution reference panels (PML Microbiologicals, Wilsonville, Ore) in ambient air and Etest strips along with disc diffusion (Becton Dickinson, Sparks, Md) in a 5% CO_2 atmosphere according to NCCLS guidelines and Etest recommendations.[1,2,8]
- A common inoculum for the panels, discs, and Etest strips was made with an overnight culture of the organism using Mueller-Hinton Broth or 0.9% physiologic saline to a turbidity equal to a 0.5 McFarland Standard. Random colony counts were used to insure inoculum uniformity.
- Broth microdilution panels to determine MIC
  - Were incubated at 35°C in ambient air for 20–24 hours.
- Etest strips to determine MIC and antibiotic discs to determine inhibition zone sizes
  - A single, 150-mm MHASB plate was used to perform susceptibility testing using Etest strips and antibiotic discs for each individual strain of *S pneumoniae* and *S pyogenes* (Figure 1).
  - A single, 150-mm HTM plate was used to perform susceptibility testing using Etest strips and antibiotic discs for each individual strain of *H influenzae* (Figure 1).
  - All cultures were incubated at 35°C in CO_2 for 20–24 hours.
- Quality control of panels and discs was performed using *S pneumoniae* ATCC 49619 and *H influenzae* ATCC 49247 on each day of testing according to NCCLS guidelines.

FIGURE 1. AGAR PLATE TEMPLATE FOR ETEST STRIPS AND ANTIBIOTIC DISCS. ONE PLATE WAS USED FOR EACH INDIVIDUAL STRAIN OF EACH ORGANISM.



RESULTS

Study organisms

- A total of 400 isolates were tested at each of the 7 laboratories involved in this study (Table 1).

TABLE 1. STUDY ORGANISMS BY GENUS, SPECIES, AND STRAIN PER LABORATORY

Genus/species, strain	Total strains (N)	Total isolates (N)
Haemophilus influenzae	50	100
Streptococcus pneumoniae	100	200
Streptococcus pyogenes	50	100
Quality control organisms		
Haemophilus influenzae ATCC 49247	10	n/a
Streptococcus pneumoniae ATCC 49617	10	n/a

Abbreviations: n/a, not applicable; ATCC, American Type Culture Collection.

Proposed MIC breakpoints

- Comparison of telithromycin MICs determined by broth microdilution and Etest support the following proposed Etest MIC breakpoints (Table 2)
  - *Streptococcus pneumoniae:* ≤4/8/≥16 mcg/mL
  - *Haemophilus influenzae:* ≤8/16/≥32 mcg/mL
  - *Streptococcus pyogenes:* ≤0.5/1/≥2 mcg/mL

TABLE 2. NCCLS-DEFINED,[a] ETEST-DEFINED, AND PROPOSED ETEST MIC BREAKPOINTS AND NCCLS-DEFINED DISC DIFFUSION ZONE BREAKPOINTS

	Streptococcus pneumoniae		*Haemophilus influenzae*		*Streptococcus pyogenes*	
Drug	MIC (mcg/mL) ≤S/I/≥R	Disc (mm) ≤R/I/≥S	MIC (mcg/mL) ≤S/I/≥R	Disc (mm) ≤R/I/≥S	MIC (mcg/mL) ≤S/I/≥R	Disc (mm) ≤R/I/≥S
TEL						
NCCLS	1/2/4	15/16–18/19	4/8/16	11/12–14/15	0.5/1/2[b]	16/18–20/21[b]
Etest	4/8/16[c]	n/a	8/16/32[c]	n/a	1/2/4[c]	n/a
AZI						
NCCLS	0.5/1/2	13/14–17/18	4/–/–[d]	–/–/12[d]	0.5/1/2	13/14–17/18
Etest	4/8/16[e]	n/a	8/–/–[d,e]	n/a	2/4/8[c]	n/a

Abbreviations: AZI, azithromycin; I, Intermediate; MIC, minimum inhibitory concentration; n/a, not applicable; NCCLS, National Committee for Clinical Laboratory Standards; R, resistant; S, susceptible; TEL, telithromycin.
[a]Breakpoints are defined in NCCLS document M100-S14, 2004, unless otherwise noted.
[b]Breakpoints as defined by the French Society of Microbiology. *Int J Antimicrob Agents.* 2003;21:364–391.[10]
[c]Proposed breakpoints based on data generated during this study.
[d]Criteria are defined for susceptibility only.
[e]Etest published breakpoints.[11]

- Susceptibility QC ranges established by NCCLS for telithromycin and azithromycin and by Etest for azithromycin are presented in Table 3.

TABLE 3. NCCLS AND ETEST SUSCEPTIBILITY QUALITY CONTROL RANGES

	Streptococcus pneumoniae ATCC 49619		*Haemophilus influenzae* ATCC 49247	
Drug	MIC (mcg/mL)	Zone size (mm)	MIC (mcg/mL)	Zone size (mm)
NCCLS				
Telithromycin	0.004–0.03	27–33	1–4	17–23
Azithromycin	0.06–0.25	19–25	1–4	13–21
Etest (CO_2)[a]				
Azithromycin	0.5–2	n/a	4–16	n/a

Abbreviations: ATCC, American Type Culture Collection; n/a, not applicable; NCCLS, National Committee for Clinical Laboratory Standards.
[a]Breakpoints established by AB Biodisk, 1998.

Activity of telithromycin and azithromycin

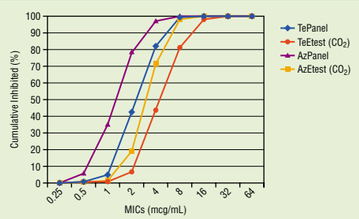
- A total of 2769/2800 (99%) tests were completed at the 7 laboratories.
- *Haemophilus influenzae*
  - Telithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} values of 4 mcg/mL and 8 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 8 mcg/mL and 16 mcg/mL, respectively (Table 4), for a difference of +1 $\log_2$ dilution between the 2 testing modalities (Figure 2).
  - Azithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} of 2 mcg/mL and 4 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 4 mcg/mL and 8 mcg/mL, respectively (Table 4), for an average difference of +1.05 $\log_2$ dilution between the 2 testing modalities (Figure 2).

TABLE 4. IN VITRO ACTIVITY OF TELITHROMYCIN AND AZITHROMYCIN AGAINST 200 STRAINS (400 ISOLATES) OF *HAEMOPHILUS INFLUENZAE*, *STREPTOCOCCUS PNEUMONIAE*, AND *STREPTOCOCCUS PYOGENES* COMPARING BROTH MICRODILUTION MICs TO ETEST MICs INDEPENDENTLY TESTED IN 7 LABORATORIES

	Broth microdilution panel in ambient air			Etest in 5% CO_2			Log_2 dilution differences: Etest vs panel		
	MIC_{50}	MIC_{90}	GeoMean	MIC_{50}	MIC_{90}	GeoMean	MIC_{50}	MIC_{90}	GeoMean
***Haemophilus influenzae* (n=690)**									
TEL	4	8	3.226	8	16	6.470	1	1	1.00
AZI	2	4	1.776	4	8	4.313	0	2	1.21
***Streptococcus pneumoniae* (n=1385)**									
TEL	0.015	0.5	0.040	0.06	4	0.164	2	3	2.05
AZI	1	128	3.299	8	>256[a]	16.779	3	2[a]	2.54
***Streptococcus pyogenes* (n=694)**									
TEL	0.015	0.03	0.023	0.06	0.12	0.082	2	2	1.78
AZI	0.5	1	0.467	2	4	2.805	2	2	3.00

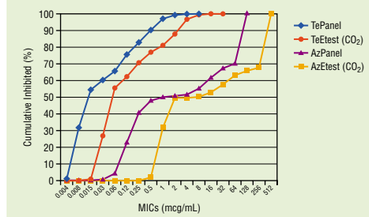
Abbreviations: AZI, azithromycin; CO_2, carbon dioxide; MIC_{50}, minimum inhibitory concentration at which 50% of the isolates were inhibited; MIC_{90}, minimum inhibitory concentration at which 90% of the isolates were inhibited; n, total number of tests completed; TEL, telithromycin.
[a]Etest MIC of >256 mcg/mL is considered to be 512 mcg/mL for purposes of calculating the $\log_2$ differences.

FIGURE 2. *HAEMOPHILUS INFLUENZAE* WITH TELITHROMYCIN AND AZITHROMYCIN CUMULATIVE PERCENTAGE INHIBITED AT EACH MIC COMPARING BROTH MICRODILUTION IN AMBIENT AIR WITH ETEST® IN 5% CO_2.



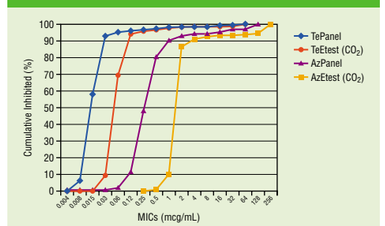
- *Streptococcus pneumoniae*
  - Telithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} values of 0.015 mcg/mL and 0.5 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 0.06 mcg/mL and 4 mcg/mL, respectively (Table 4), for an average difference of +2.5 $\log_2$ dilutions between the 2 testing modalities (Figure 3).
  - Azithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} values of 1 mcg/mL and 128 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 8 mcg/mL and >256 mcg/mL, respectively (Table 4), for an average difference of +2.39 $\log_2$ dilution between the 2 testing modalities (Figure 3).

FIGURE 3. *STREPTOCOCCUS PNEUMONIAE* WITH TELITHROMYCIN AND AZITHROMYCIN CUMULATIVE PERCENTAGE INHIBITED AT EACH MICCOMPARING BROTH MICRODILUTION IN AMBIENT AIR WITH ETEST® IN 5% CO_2.



- *Streptococcus pyogenes*
  - Telithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} values of 0.015 mcg/mL and 0.03 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 0.06 mcg/mL and 0.12 mcg/mL, respectively (Table 4), for an average difference of +1.95 $\log_2$ dilutions between the 2 testing modalities (Figure 4).
  - Azithromycin: broth microdilution in ambient air produced MIC_{50} and MIC_{90} values of 0.5 mcg/mL and 1 mcg/mL, respectively, compared with Etest values in 5% CO_2 of 2 mcg/mL and 4 mcg/mL, respectively (Table 4), for an average difference of +2.5 $\log_2$ dilutions between the 2 testing modalities (Figure 4).

FIGURE 4. *STREPTOCOCCUS PYOGENES* WITH TELITHROMYCIN AND AZITHROMYCIN CUMULATIVE PERCENTAGE INHIBITED AT EACH MIC COMPARING BROTH MICRODILUTION IN AMBIENT AIR WITH ETEST® IN 5% CO_2.



Quality control

- The frequency distribution of MICs for the QC organisms, *H influenzae* ATCC 49247 and *S pneumoniae* ATCC 49619, generated from broth microdilution panels in ambient air and from Etest in 5% CO_2, are presented in Table 5.
- The QC range for telithromycin against *H influenzae* was 2 $\log_2$ dilutions higher for the Etest compared with broth microdilutions (range 4–16 mcg/mL vs 1–4 mcg/mL, respectively).
- The QC range for azithromycin against *H influenzae* ATCC 49247 was 2 $\log_2$ dilutions higher for the Etest compared with broth microdilutions (range 4–16 mcg/mL vs 1–4 mcg/mL, respectively).
- The QC range for telithromycin against *S pneumoniae* ATCC 49619 was 1 $\log_2$ dilution higher for the Etest compared with broth microdilutions (range 0.03–0.06 mcg/mL vs 0.008–0.03 mcg/mL, respectively).
- The QC range for azithromycin against *S pneumoniae* ATCC 49619 was 2 $\log_2$ dilutions higher for the Etest compared with broth microdilutions (range 0.5–2 mcg/mL vs 0.12–1 mcg/mL, respectively).

TABLE 5. FREQUENCY DISTRIBUTION OF MIC VALUES FOR QUALITY CONTROL ORGANISMS COMPARING BROTH MICRODILUTION IN AMBIENT AIR TO ETEST IN 5% CO_2[a]

		MIC (mcg/mL)															Log_2 shift in modal value
Organism	Drug	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16				
Haemophilus influenzae ATCC 49247 (n=418)	TEL (Panel), n								1	37[b]	32						
	TEL (Etest), n[c]										23	46[b]	1				2+
	AZI (Panel), n								8	46[b]	16						
	AZI (Etest), n										16	50[b]	4				2+
Streptococcus pneumoniae ATCC 49619 (n=418)	TEL (Panel), n	31	36[b]	3													
	TEL (Etest), n[d]			46[b]	24												1+
	AZI (Panel), n					24	37[b]	6	1								
	AZI (Etest), n							2	42[b]	26							2+

Abbreviations: AZI, azithromycin; MIC, minimum inhibitory concentration; n, number of tests; TEL, telithromycin.
[a]National Committee for Clinical Laboratory Standards–approved quality control ranges are shaded in yellow.
[b]Proposed Etest quality control range based on data from this study: 4–16 mcg/mL.
[c]Modal value.
[d]Proposed Etest quality control range based on data from this study: 0.015–0.12 mcg/mL.

CONCLUSIONS

- Telithromycin MICs are 1–2 $\log_2$ dilutions higher when testing fastidious organisms by Etest in CO_2 compared with NCCLS reference broth microdilution.
- Azithromycin MICs are 2–3 $\log_2$ dilutions higher when testing fastidious organisms by Etest in CO_2 compared with NCCLS reference broth microdilution.
- Therefore, the Etest methodology is not recommended for the routine in vitro testing of telithromycin for *H influenzae*, *S pneumoniae*, and *S pyogenes*.
- If, however, the Etest methodology is used to determine telithromycin MIC for these pathogens, the appropriate $\log_2$ correction factors should be applied by the laboratory before reporting these Etest MICs or interpretive susceptibilities for fastidious organisms to clinical physicians.

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