ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2005, p. 464–466 Vol. 49, No. 1
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.1.464–466.2005

Influence of Carbon Dioxide on the MIC of Telithromycin for *Streptococcus pneumoniae*: an In Vitro-In Vivo Study

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Received 24 April 2004/Returned for modification 23 June 2004/Accepted 10 September 2004

Incubation in CO_2 resulted in higher (≥3 doubling dilution) MICs of telithromycin than those found in ambient air for 31.2% of 346 *Streptococcus pneumoniae ermB*-positive strains. An increased telithromycin MIC in CO_2 was not correlated with loss of its activity in the murine sepsis/peritonitis model.

Incubation in CO_2 has been reported to result in higher MICs of macrolides, clindamycin, and telithromycin for *Streptococcus pneumoniae* than those found in ambient air (6, 9, 10, 11, 15, 16). Conversely, susceptibility testing of other antibiotics, namely β-lactams, evernimicin, and linezolid, does not appear to be affected by the presence or absence of CO_2 (3, 5, 7, 8). This study reports the influence of CO_2 on the MIC of telithromycin against *ermB*-positive *S. pneumoniae*.

MICs of erythromycin and telithromycin in ambient air and CO_2. MICs of erythromycin and telithromycin were determined by using the agar dilution method for 675 clinical strains of *S. pneumoniae* (13). Plates were incubated for 16 h at 37°C, either in ambient air or in 5% CO_2. Susceptibility to erythromycin was also assessed by agar diffusion (15 μg of erythromycin per disk). MICs of telithromycin were also measured by microdilution according to the NCCLS recommendations (13). The MIC breakpoints of erythromycin were defined as the following: susceptible, ≤0.25 mg/liter; resistant, ≥1 mg/liter (13). The MIC breakpoints of telithromycin were defined according to the European Agency for the Evaluation of Medicinal Products (susceptible, ≤0.5 mg/liter; resistant, >2 mg/liter) and according to the proposed NCCLS breakpoints (susceptible, ≤1 mg/liter; resistant, ≥4 mg/liter).

Erythromycin MIC in ambient air was ≥0.25 mg/liter for 345 strains. Ten additional strains for which the erythromycin MICs were ≤0.25 mg/liter had an inhibition zone diameter of <22 mm. These 355 strains were tested for *ermB* and *mefE* genes by PCR as previously described (2). Erythromycin resistance genes were distributed as follows: *ermB* was present in 347 strains, *mefE* in 4 strains, *mefE* and *ermB* in 2 strains, and neither *ermB* nor *mefE* in 2 strains. Telithromycin MICs in ambient air (675 strains) were distributed as follows: ≤0.5 mg/liter for 667 strains (98.8%), 1 mg/liter for 4 strains (0.6%), and 2 mg/liter for 4 strains (0.6%). Telithromycin MICs were obtained in CO_2 for 659 strains and were ≤0.5 mg/liter for 591 strains (89.7%), 1 mg/liter for 36 strains (5.5%), and 2 mg/liter for 32 strains (4.8%). According to the European breakpoints, the frequency of strains with intermediate susceptibility to telithromycin was 1.2% in ambient air and 10.3% in CO_2. These frequencies, according to the NCCLS breakpoints, were 0.6 and 4.9%, respectively.

For 346 *ermB*-positive strains, the telithromycin MIC at which 50% of isolates tested are inhibited (MIC_{50}) in ambient air and in CO_2 was 0.015 and 0.06 mg/liter, and the MIC_{90} was 0.25 and 2 mg/liter, respectively (Fig. 1). The proportion of strains for which the telithromycin MIC was >0.5 mg/liter was 2.3% in ambient air and 21.5% in CO_2 ($P < 0.001$). The proportion of strains for which the telithromycin MIC was >1 mg/liter was 1.2% in ambient air and 11.5% in CO_2 ($P < 0.001$). The distribution of the CO_2/ambient air (CO_2/AA) ratio is shown for 346 *ermB*-positive strains and for 310 erythromycin-susceptible or *mefE* strains in Fig. 2. The median CO_2/AA ratio of *ermB*-positive strains was 2 $\log_2$ dilutions (range, −1 to +8). For these 346 strains, the CO_2/AA ratio was ≥3 $\log_2$ dilutions for 109 strains (31.5%), ≤1 $\log_2$ dilutions for 141 strains (40.7%), and 2 $\log_2$ dilutions for 96 strains (27.7%). According to the European breakpoints, 71 strains out of 346 (20.5%) were classified in different categories for incubation in ambient air and CO_2: 64 were susceptible in ambient air and intermediate in CO_2, 6 were intermediate in ambient air and resistant in CO_2, and 1 strain was susceptible in ambient air and resistant in CO_2. Using the NCCLS breakpoints, 38 strains out of 346 (11.0%) were classified in different categories in ambient air and in CO_2: 31 were susceptible in ambient air and intermediate in CO_2, 4 were intermediate in ambient air and resistant in CO_2, and 3 were susceptible in ambient air and resistant in CO_2.

Reproducibility. Twenty strains for which the telithromycin MIC in CO_2:MIC in ambient air ratios (CO_2/AA ratio) was ≤1 $\log_2$ dilution were randomly selected from the 346 *ermB*-positive strains (Group A), as were 20 other strains for which the telithromycin CO_2/AA ratio was ≥3 $\log_2$ dilutions (Group B). In order to assess its reproducibility, telithromycin MICs were replicated 10 times by using the agar dilution method as described above, both in ambient air and in 5% CO_2. MIC reproducibility was expressed as the standard deviation (SD) of $\log_2$ MICs for each strain. The SDs for the MICs were calculated after $\log_2$ transformation of MICs to express them in doubling dilutions. The SDs of the telithromycin MIC were ≤1

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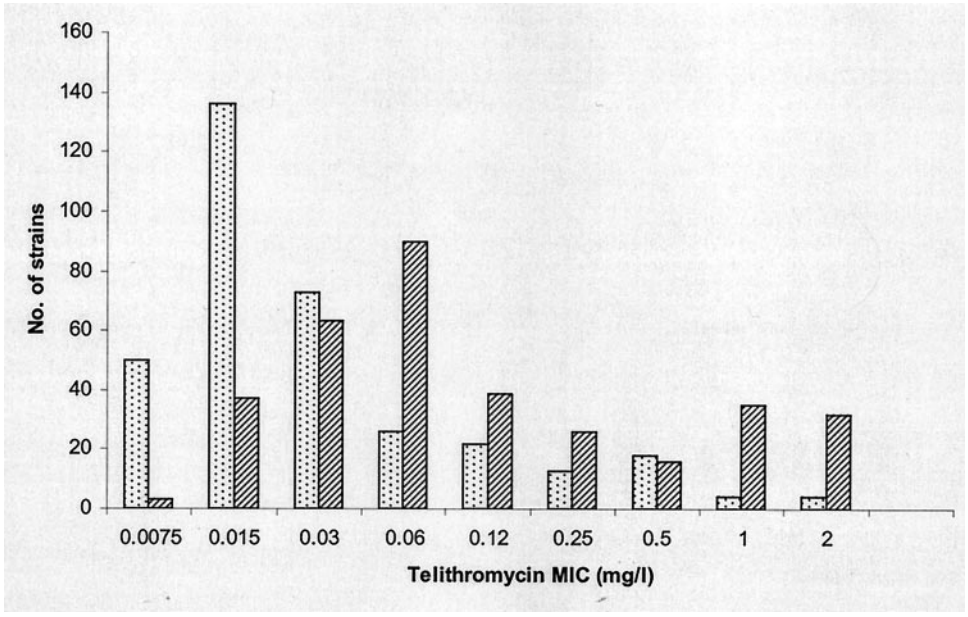

FIG. 1. Distribution of telithromycin MICs measured in ambient air (light bars) and in 5% CO_2 (dark bars) for 346 *ermB*-positive strains of *S. pneumoniae*.

$\log_2$ dilution for 31 strains in ambient air (range, 0.0 to 2.0) and for 36 strains in CO_2 (range, 0.0 to 1.2). The 10 determinations of the MIC were within ≤3 doubling dilutions for 29 strains in ambient air and for 32 strains in CO_2. The mean CO_2/AA ratios ranged from 0.5 to 2.0 doubling dilutions in Group A and from 1.9 to 6.2 doubling dilutions in Group B. The SD of the CO_2/AA ratio was ≤1 $\log_2$ dilutions for 36 out of 40 strains (range, 0.0 to 1.8).

Experimental murine sepsis/peritonitis model. Among the 40 erythromycin-resistant strains tested in the reproducibility assay, 8 strains were selected for in vivo experiments: 3 strains for which the telithromycin MIC was ≤0.5 mg/liter in ambient air and >0.5 mg/liter in CO_2 (strains C05SP06, C08SP13, and C04SP28); 2 strains for which the MICs were ≤0.5 mg/liter in both atmospheres (strains C01SP07 and C22SP03); and 3 strains for which the MICs were >0.5 mg/liter in both atmospheres (C28SP22, C18SP04, and C19SP10). The inoculum was obtained as follows. Bacterial suspensions were prepared from 16-h cultures at 37°C in ambient air on 5% sheep blood agar plates. The inoculum was adjusted to 6 MacFarlands and then was diluted at equal parts with a 10% mucin saline solution (M-2378; Sigma, St. Quentin-Fallavier, France). To quan-

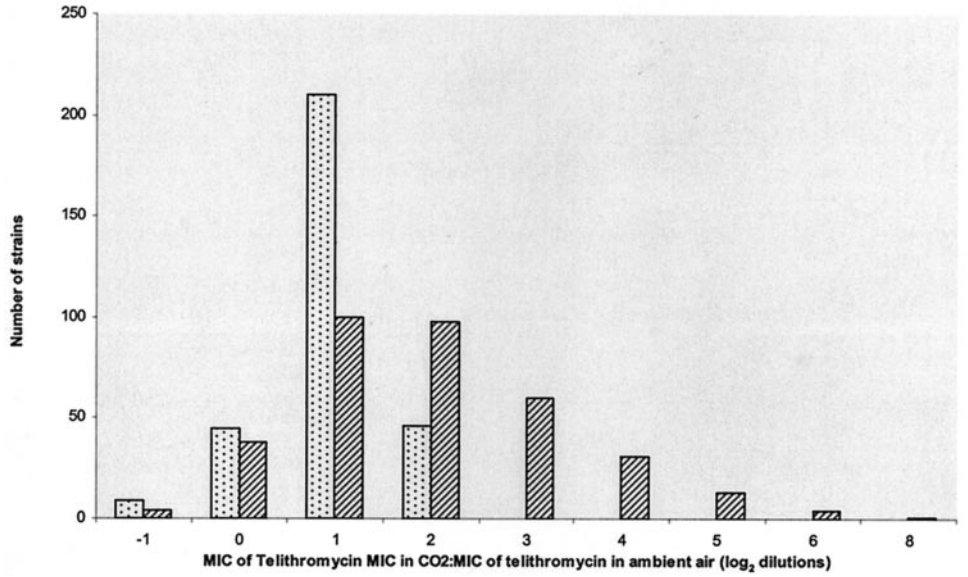

FIG. 2. Distribution of the CO_2/AA ratio for 346 *ermB*-positive strains of *S. pneumoniae* (dark bars) and for 310 erythromycin-susceptible or *mefA*-positive strains (light bars).

TABLE 1. MICs and ED_{60}s determined in this study

Strain	Agar dilution MIC		CO_2/AA ratio	Microdilution MIC	ED_{50}
	Ambient air	5% CO_2			
C01SP07	0.01 ± 0.7	0.05 ± 0.5	1.5 ± 0.7	0.015	0.6
C22SP03	0.04 ± 0.5	0.12 ± 0.7	1.2 ± 0.8	0.03	0.6
C05SP06	0.04 ± 1.1	0.50 ± 1.2	3.5 ± 0.7	0.03	0.6
C08SP13	0.06 ± 1.4	0.93 ± 0.8	4.0 ± 0.9	0.5	0.3
C04SP28	0.03 ± 0.4	0.78 ± 1.2	4.4 ± 1.2	0.06	0.9
C28SP22	1.62 ± 0.5	3.53 ± 0.0	1.3 ± 0.5	2	17.1
C18SP04	0.93 ± 0.3	3.31 ± 0.3	2.0 ± 0.5	1	18.8
C19SP10	1.00 ± 0.0	3.31 ± 0.3	1.9 ± 0.3	1	12.5

tify the inoculum, 50 μl of the final suspension was plated on sheep blood agar plates after appropriate dilutions, and colonies were counted after a 24-h incubation on sheep blood agar at 37°C in a 5% CO_2 atmosphere. Female 28- to 30-g Swiss mice (Elevage Janvier SA, Le Genest St. Isle, France) were kept in cages with free access to food and water. Mice were injected intraperitoneally with 0.7 ml of a fresh suspension. Telithromycin was administered subcutaneously at increasing concentrations (volume, 0.5 ml) immediately and 4 h after inoculation. For each strain, 10 mice were inoculated at each telithromycin dose level, which ranged from 0.01 to 100 mg/kg of body weight, expressed as a single dose. Mice were observed for 6 days and then were sacrificed. This protocol was approved by the University of Nantes experimental therapeutic unit. The median effective dose (ED_{50}) was calculated as previously described by Reed and Muench (14). Mean ED_{50}s were compared using the Mann-Whitney test. The mean inoculum was 10^7 CFU per mouse (range, 3×10^6 to 5×10^7 CFU per mouse). All nontreated control mice died within 48 h after inoculation. ED_{50}s of the five strains susceptible to telithromycin (geometric mean telithromycin MIC, ≤0.5 mg/liter in ambient air) were almost identical (mean ED_{50}, 0.6 ± 0.2 mg/kg), regardless of whether the MIC determined in CO_2 was below or above 0.5 mg/liter (Table 1). Conversely, telithromycin ED_{50}s were higher for the three strains for which the geometric mean telithromycin MIC was >0.5 mg/liter in ambient air (mean ED_{50}, 16.1 ± 3.3 mg/kg; $P < 0.05$).

This study demonstrates that for 346 *ermB*-positive strains of *S. pneumoniae*, the telithromycin MICs are higher in CO_2 than in ambient air. Although the telithromycin MICs for 2.3% of strains were >0.5 mg/liter in ambient air, this proportion increased to 21.5% in CO_2. It should be noted that CO_2 does not influence the telithromycin MIC in the same way for all strains of *S. pneumoniae*, as the MICs for 31.5 and 40.7% of the 346 *ermB*-positive strains increased to ≥3 and ≤1 doubling dilution, respectively. It therefore seems possible to use the magnitude of the increase in MICs in CO_2 to classify strains of *ermB*-positive pneumococci. Our results with the murine peritonitis/septicemia model suggest that telithromycin remains effective against strains that are susceptible in ambient air (MICs ≤ 0.06 mg/liter), regardless of whether the MIC in CO_2 remains low (≤0.12 mg/liter) or increases to intermediate values (≥0.5 mg/liter).

As has already been suggested for macrolides by other studies, these data support the hypothesis that it may be relevant to determine different breakpoints depending on whether the telithromycin susceptibility test is performed in ambient air or in CO_2 (6, 9, 15). The British Society for Antimicrobial Chemotherapy, the NCCLS, and the French Society of Microbiology recommend that agar dilution be performed in CO_2 for *S. pneumoniae* (1, 4, 12, 13). The French Society of Microbiology has added to its 2003 recommendations that resistance to telithromycin in CO_2 should be confirmed in ambient air (12).

We are grateful to Anne-Françoise Miègeville and Virginie Le Mabecque for technical assistance.

This work was supported in part by Aventis Laboratories, Paris, France.

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