

Effect of pH on the In Vitro Potency of Clarithromycin against *Mycobacterium avium* Complex

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Employing 7H11 agar medium at pH 6.6, the MICs of clarithromycin for 50% (MIC₅₀) and 90% (MIC₉₀) of 19 strains of *Mycobacterium avium* complex were 8 and 16 µg/ml, respectively. However, the MICs were 2 to 3 log₂ dilutions lower in the 7H11 medium adjusted to pH 7.4, and the MICs on 10% OADC (oleic acid-albumin-dextrose-catalase)-enriched Mueller-Hinton agar at pH 7.3 were also 2 log₂ dilutions lower than those measured on 7H11 agar at pH 6.6. Therefore, clarithromycin is more active at a physiologic than at an acidic pH.

Clarithromycin, a new macrolide, exhibits a broad antibacterial spectrum and is active against clinically important gram-positive and gram-negative organisms. In vitro, the drug appears as potent as or 1 or 2 log₂ dilutions more potent than erythromycin (1-3); in addition, greater concentrations of clarithromycin are achieved in serum and tissues than are achieved by equivalent doses of erythromycin (4).

Employing Bacto Mycobacteria 7H11 agar, final pH 6.6 (Difco Laboratories, Detroit, Mich.), we determined the MICs of clarithromycin against 19 strains of *Mycobacterium avium* complex, all isolated from patients with AIDS. Organisms were grown for 14 days on 7H11 agar medium enriched with 10% (vol/vol) OADC (oleic acid, albumin, dextrose, and catalase; Difco Laboratories). Transparent colonies were collected and subcultured for 7 days in Dubos medium (Diagnostics Pasteur, Paris, France), and the suspensions were adjusted to match the turbidity of *M. bovis* BCG standard (1 mg/ml). We then diluted the adjusted suspensions to concentrations of 10⁻³ and 10⁻⁵ mg/ml and plated each suspension onto 10% OADC-enriched 7H11 agar quadrant plates (0.05 ml per quadrant). Concentrations of clarithromycin ranging from 0.5 to 64 µg/ml were added to the agar plates. The plates were incubated for 14 days at 37°C, and the MIC was defined as the lowest concentration of clarithromycin which inhibited at least 99% of growth compared with drug-free agar plates. The MICs for 50% (MIC₅₀) and 90% (MIC₉₀) of the strains were 8 and 16 µg/ml, respectively; these values are similar to those obtained employing 7H10 (4) or 7H11 agar (8) or 7HSF broth (6), which is equivalent to 7H11 agar without malachite green. Because pharmacokinetic studies in humans indicated that the concentration of clarithromycin in serum was only 4 µg/ml after the administration of 1,000 mg of clarithromycin (Abbott Laboratories) twice daily, it appeared that clarithromycin was unlikely to be useful in the treatment of infection caused by *M. avium* complex.

However, macrolides are known to be more potent at a basic pH than at an acidic pH (5, 7). This has also been shown to be the case when clarithromycin was tested against organisms other than *Mycobacteria* spp. (1, 3). It may be, therefore, that the MIC of clarithromycin against *M. avium* complex at a physiologic pH is considerably smaller than that observed at an acidic pH. To test this hypothesis, we

compared the MICs of clarithromycin against four strains of *M. avium* complex, employing standard 7H11 agar medium at pH 6.6, prepared by rehydrating the dehydrated medium with distilled water as recommended by the manufacturer, and the same agar medium adjusted to pH 7.4, prepared by rehydrating the dehydrated medium with phosphate buffer of pH 7.5. The MICs of clarithromycin for the four strains on agar at pH 6.6 were 8, 4, 8, and 4 µg/ml, whereas the corresponding MICs at pH 7.4 were 2, 1, 1, and ≤0.5 µg/ml, indicating that the MICs of clarithromycin at pH 7.4 were 2 to 3 log₂ dilutions lower than those at pH 6.6.

It may not be justified to modify the pH of a medium that has been prepared as a buffered powder. Because our purpose was to determine the MICs of clarithromycin at a physiologic pH, we compared the MICs of clarithromycin against 20 strains of *M. avium* complex employing standard 7H11 and 10% OADC-enriched Bacto Mueller-Hinton agar (Difco Laboratories) media. The reasons we selected Mueller-Hinton agar were (i) its final pH was 7.3 when prepared by procedures recommended by the manufacturer, and (ii) we have observed that the CFU of bacterial suspensions of four strains of *M. avium* complex on 7H11 agar were 8.57, 8.29, 6.57, and 8.29 log₁₀/ml, whereas the corresponding values obtained from 10% OADC-enriched Mueller-Hinton agar were 8.57, 8.18, 6.68, and 8.24 log₁₀/ml, indicating that *M. avium* complex multiplies as well on 10% OADC-enriched Mueller-Hinton agar medium as on 7H11 agar. As indicated by the data of Table 1, the MICs on OADC-enriched Mueller-Hinton agar medium were again 2 log₂ dilutions lower than those measured on 7H11 agar medium. In a separate experiment employing OADC-enriched Mueller-Hinton agar medium, the MIC₉₀ of erythromycin against 18 strains of *M. avium* complex was >64 µg/ml, a concentration very much higher than the achievable peak level in human serum.

Because of a number of complicating factors, it is difficult to predict the in vivo activity of a macrolide against *M. avium* complex on the basis of results in vitro. The organisms reside within the cells, where the pH may be significantly lower than 7.4. Moreover, macrolides are known to be concentrated intracellularly. Therefore, the clinical relevance of our observation that clarithromycin is more active at a physiologic than an acidic pH is not entirely clear. Nevertheless, the fact that the MICs of clarithromycin against *M. avium* complex lie within the range of achievable

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TABLE 1. MICs of clarithromycin against 20 strains of *M. avium* complex on 7H11 agar and 10% OADC-enriched Mueller-Hinton agar

Medium (pH)	MIC ($\mu\text{g/ml}$) ^a		
	Range	50%	90%
7H11 (6.6)	2-32	8	16
Mueller-Hinton (7.3)	0.5-8	2	4

^a 50 and 90%, MIC for 50 and 90% of isolates tested, respectively.

levels in serum suggests that a clinical trial of clarithromycin in infections caused by *M. avium* complex is warranted.

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