MACROLIDE RESISTANCE IN *MYCOBACTERIUM AVIUM*

by

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(ABSTRACT)

Mycobacterium avium isolates resistant to clarithromycin and azithromycin have been recovered from patients undergoing antibiotic therapy. Comparison of DNA fingerprints of sensitive and resistant isolates showed that resistance resulted from mutation of the original, sensitive isolate in five of seven patients. In the other two patients, the clarithromycin-resistant isolates were unrelated to the sensitive isolate, suggesting that the resistant isolate resulted from either superinfection or selection of a resistant strain from a polyclonal population.

Investigation of the mechanisms of clarithromycin and azithromycin resistance in *M. avium* showed that high-level resistance resulted from a point mutation at position A-2058 in the 23S rRNA. Based on this finding, a rapid screen for clarithromycin-resistance in *M. avium* was developed based on PCR. Twenty-three clinical isolates were analyzed, seven of which were clarithromycin-resistant. The target product was amplified only in clarithromycin-resistant strains, all of which had mutations at position 2058.

A polyuridylic acid (poly U)-dependent *in vitro* translation system from *M. avium* was developed to investigate the effect of antibiotics on protein synthesis. Clarithromycin was an effective inhibitor of protein synthesis in cell-free extracts of a susceptible *M. avium* strain, whereas a high-level resistant strain was less susceptible to clarithromycin *in vitro*. Mixtures of extracts from sensitive and resistant strains showed a pattern of clarithromycin inhibition similar to the resistant strain, suggesting that resistance may be dominant in partial diploids.

Three *M. avium* strains exhibiting step-wise, intermediate resistance to azithromycin were characterized in comparison to the sensitive parent. All strains were similar in hydrophobicity, growth medium requirements, and growth response to temperature. The azithromycin-resistant strains were resistant to several unrelated agents, including ciprofloxacin, rifabutin, and ethidium bromide. Addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) did not lower minimal inhibitory concentrations (MICs) for ciprofloxacin or ethidium bromide. Cell-free extracts of the strains were as sensitive to azithromycin *in vitro* as the parent strain. The results rule out inactivation, efflux, and mutations in the target as resistance mechanisms, and suggest intermediate resistance may be due to altered permeability of the cell wall or membrane.