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Genetic Basis for Clarithromycin Resistance among Isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus*

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Resistance to clarithromycin among isolates of *Mycobacterium chelonae* and *M. abscessus* was observed in 18 of 800 (2.3%) patients tested between 1990 and 1995. Patients whose isolates were resistant had either disseminated disease or chronic lung disease, and the resistant isolates were recovered after clarithromycin monotherapy. Sequencing of the gene coding for the 23S rRNA peptidyltransferase region revealed a point mutation involving adenine at position 2058 (38%) or adenine at position 2059 (62%) in 20 of 20 relapse isolates from the first 13 patients identified. By pulsed-field gel electrophoresis or random amplified polymorphic DNA PCR, initial and relapse isolates were shown to have identical DNA patterns. *M. chelonae* and *M. abscessus* isolates were found to have only a single chromosomal copy of the rRNA operon, thus making them susceptible to single-step mutations. Thus, clarithromycin resistance in these species of rapidly growing mycobacteria relates to a point mutation in the gene coding for 23S rRNA and occurs in limited clinical situations, but was identified in almost 5% of isolates tested in 1995.

The newer macrolides have had a dramatic impact on the success of therapy for diseases caused by nontuberculous mycobacteria. In the laboratory, MICs for almost all species are low (3, 5), and successes in clinical trials have been recorded with azithromycin for disseminated *Mycobacterium avium* complex disease (17, 38) and with clarithromycin for *M. avium* complex infection in the settings of both disseminated disease in patients with AIDS (6, 10, 11) and chronic lung disease in patients without AIDS (9, 13, 33). Treatment successes with clarithromycin against *M. xenopi* (8), *M. marinum* (7), *M. haemophilum* (16), *M. genavense* (2, 23), *M. chelonae* (36), and *M. abscessus* (18, 22) have also been reported in case reports and one controlled trial. These success stories have been tempered by reports of the development of drug resistance, especially when the drug was given as monotherapy (6, 10, 31, 33, 36). Studies with *M. intracellulare* (20) and *M. avium* (21, 24) have shown that the clarithromycin resistance that develops in vivo in these two species is associated with a point mutation at either the adenine at position 2058 (A2058) or A2059 in the peptidyltransferase region of the 23S rRNA gene. For isolates with these mutations, clarithromycin MICs increased from ≤4 to ≥128 μg/ml and the isolates were cross resistant to azithromycin (14, 24). This molecular mechanism of macrolide resistance is a first among bacterial pathogens, because previously identified resistance to this class of drugs has involved the *erm* genes, enzymes which modify A2058 in 23S rRNA in *trans* by dimethylation. It has been hypothesized that this unique mechanism in *M. avium* acting in *cis* is due to the fact that *M. avium* and most other slowly growing mycobacteria, in contrast to most bacterial pathogens, harbor a single, chromosomal rRNA operon (1, 12, 24).

Similar genetic studies of other nontuberculous mycobacteria which have become resistant to clarithromycin have not been done, although clinical reports of such resistance have been published (7, 31, 35). We routinely determine the clarithromycin susceptibilities of species of nontuberculous mycobacteria other than the *M. avium* complex. In this regard, it is particularly interesting to study rapidly growing mycobacteria because these organisms harbor plasmids and antibiotic-modifying enzymes (25, 34) and are believed to possess two rRNA operons (1). We identified within the laboratory isolates of rapidly growing mycobacteria resistant to clarithromycin, obtained clinical data on the patients from whom the organisms were isolated, and investigated whether the mechanism of clarithromycin resistance was the same as that observed in organisms of the *M. avium* complex.

MATERIALS AND METHODS

Organisms. Clinical isolates of *M. chelonae* and *M. abscessus* submitted to the Mycobacteria/Nocardia Reference Laboratory of the University of Texas Health Center at Tyler were screened for isolates for which clarithromycin MICs were >4 μg/ml by using Mueller-Hinton broth at pH 7.4 (5). Clinical information was obtained on the patients from whom the organisms were isolated, and pretreatment and relapse isolates were taken from frozen stocks, maintained at −70°C in Trypticase soy agar with 15% glycerol, and subjected to genetic evaluation. Organisms had initially been identified as belonging to the *M. chelonae* group by outside laboratories. Identification of the organisms within the *M. chelonae* group (*M. chelonae* and *M. abscessus*) was done by determining their utilization of citrate (27) and differences in their susceptibilities to cefoxitin and tobramycin (29).

Clarithromycin susceptibilities. Broth microdilution plates were prepared by using serial twofold dilutions of clarithromycin, cefoxitin, tobramycin, and other antimicrobial agents, which were added to cation-supplemented Mueller-Hinton II broth (pH 7.4) as described previously (5). The final inoculum size was 10^4 CFU per well, as determined by the method of Swenson et al. (29). MICs were defined as the lowest concentration that completely inhibited macroscopic growth after incubation at 30°C for 72 h.

PCR and sequencing of the gene encoding for the 23S rRNA peptidyltransferase region. Isolates for testing were grown simultaneously on Middlebrook 7H10 agar alone, 7H10 agar with 25 or 50 μg of clarithromycin per ml, and 7H10 agar with 500 μg of clarithromycin per ml. Organisms were taken from the agar plate with the highest concentration of clarithromycin containing growth and were subjected to PCR and DNA sequencing as described previously (20, 21).

DNA typing. Analysis of large restriction fragment patterns by pulsed-field gel electrophoresis (PFGE) was performed with all available groups of isolates that included a pretreatment susceptible isolate and one or more resistant relapse

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TABLE 1. Clinical isolates of the *M. chelonae* group (*M. chelonae* and *M. abscessus*) resistant to clarithromycin by year of isolation

Year	No. of isolates tested	No. (%) resistant to clarithromycin
1990	65	0 (0)
1991	174	0 (0)
1992	181	4 (2.2)
1993	123	3 (2.4)
1994	163	2 (1.2)
1995	194	9 (4.6)
Total	800	18 (2.3)

isolates for purposes of strain comparison. For PFGE genomic DNA was prepared as described previously (19, 20, 37). Restriction endonuclease digestion was performed with *Dra*I and *Xba*I. For electrophoresis we used a CHEF-DRII system (Bio-Rad Laboratories, Richmond, Calif.) with commercial linear DNA standards as described previously (37). Previous studies have shown that approximately 50% of *M. abscessus* isolates have broken or lysed DNA after processing and cannot be studied by PFGE (37). For isolates that could not be compared by PFGE, random amplified polymorphic DNA analysis or arbitrarily primed PCR was performed with DNA extracted by boiling a cell suspension in Tris-EDTA (TE) buffer with 0.1% Triton X-100 for 30 min or by standard phenol and chloroform extraction (17a). Control strains of *M. abscessus* were included for comparison. Two previously described random primers, OPA 2 (TGCCGAGC TA) and OPA 18 (AGGTGACCGT), studied with *M. malmoense* by Kauppinen et al. (15), were used for amplification.

Southern blot analysis. Mycobacteria grown on Middlebrook 7H10 agar were heat inactivated for 10 min at 80°C, centrifuged, and resuspended in 400 μl of Tris-EDTA-Tween-lysozyme (10 mM, 10 mM, 0.1% [wt/vol], and 2 mg/ml, respectively) and were incubated for 2 h at 37°C with constant shaking. Sodium dodecyl sulfate (SDS) and proteinase K were added to final concentrations of 1% and 0.1 mg/ml, respectively. Proteins were extracted by the addition of phenol-chloroform-isoamyl alcohol (25/24/1; vol/vol/vol). After the addition of NaCl (final concentration, 125 mM), the samples were incubated on ice for 10 min and were centrifuged at 13,000 × *g* for 20 min. The DNA in the supernatant was precipitated by the addition of 2.5 volumes of ethanol and NaCl (final concentration, 225 mM). After incubation at −70°C for 30 min, the DNA was pelleted by centrifugation. The pellet was washed with 1 ml of 70% ethanol, dried, and resuspended in TE (10 mM Tris, 1 mM EDTA). Genomic DNA was digested with *Bam*HI (20 U), run on an agarose gel, transferred to a nitrocellulose membrane, and hybridized to a gene fragment spanning positions 9 to 802 of the 16S rRNA which had been generated by PCR by using *M. bovis* BCG as the template.

After labeling with the random primer DNA Labeling System (Gibco BRL) and [α-^{32}P]dCTP (Amersham), the probe was hybridized for 15 h at 65°C. The blot was washed four times for 30 min each time with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 72°C and exposed to X-ray film.

RESULTS

Organisms. Among 800 patients from whom one or more clinical isolates of the *M. chelonae* group were isolated between February 1990 and December 1995, clarithromycin susceptibility testing revealed that 784 (97.7%) were infected with isolates for which the MICs were ≤4 μg/ml. Eighteen patients (2.3%) were infected with one or more isolates for which clarithromycin MICs were >4 μg/ml. The first of these resistant isolates was identified in 1992, with an annual incidence of clarithromycin resistance of 1.2 to 4.6%. The highest incidence (4.6%) was seen in 1995 (Table 1). The resistant isolates came from six patients infected with *M. chelonae* and 12 patients infected with *M. abscessus.*

Clinical information on the patients from whom these 18 isolates were obtained is provided in Table 2. Patients had either localized lung disease (seven patients) or disseminated disease (seven patients). Patients with disseminated disease had involvement of skin, joints, or bone (three patients) or lung plus one or more sites (three patients). For four patients no history other than the isolate source was available. Five of the seven patients with disseminated disease were on corticosteroids, whereas none of the seven patients with localized lung disease was on corticosteroids. All 11 patients for whom histories were available had been on clarithromycin monotherapy prior to recovery of the resistant isolate. The clinical histories of three patients have been reported previously (30, 31, 36), with recognition of clarithromycin resistance among isolates from two of them (31, 36).

Clarithromycin susceptibilities. Among the isolates of *M. chelonae* and *M. abscessus*, clarithromycin MICs were available for 11 isolates from 9 patients prior to therapy and for 28 isolates from 18 patients after clinical relapse (Table 3). For the 11 pretreatment isolates, clarithromycin MICs were ≤0.25 μg/ml (MIC range, ≤0.063 to 0.25 μg/ml) (one additional pretreatment isolate tested in an outside laboratory was susceptible by disk diffusion testing), while for 26 of 28 relapse isolates (93%), clarithromycin MICs were ≥16 μg/ml and most isolates were resistant to the highest concentration tested (>128 μg/ml). For two relapse isolates (MC958 in Table 3), clarithromycin MICs were 0.25 and 1.0 μg/ml, respectively. Single colonies picked from the former culture (selected because the MICs for the other isolates were known to be high) revealed a mixed population, with MICs for selected colonies being >32 μg/ml.

PCR of the peptidyltransferase region of the 23S rRNA gene for gene sequencing. Sequencing of the peptidyltransferase gene region was done by using clarithromycin-susceptible pretreatment isolates from five patients. All isolates had a wild-type peptidyltransferase gene region. Sequencing was done on 20 clarithromycin-resistant relapse isolates from the first 13 patients whose isolates were identified to be clarithromycin resistant; all of the isolates had a single point mutation in the peptidyltransferase gene region. Mutations in isolates from five patients (38%) involved A2058, and mutations in isolates from eight patients (62%) involved A2059. A substitution of A → G was responsible for 10 of 13 (77%) of the point mutations.

For six patients, more than one relapse isolate obtained in culture was sequenced; cultures of samples from different sites were available for five of these patients. Despite this, only a single mutation in isolates from each patient was seen.

DNA typing. At least one pretreatment and one or more relapse isolates were available from nine patients, and the

TABLE 2. Clinical information for patients infected with *M. chelonae* or *M. abscessus* isolates resistant to clarithromycin

Underlying condition	No. of patients	No. of patient on steroids/ total no. of patients	No. of patients receiving a course of monotherapy[a]
Disseminated disease[b]			
Organ transplantation	2	2/2	2
Autoimmune disorders	3	3/3	3
AIDS	1	0/1	1
Prosthetic heart valve	1	0/1	1
Subtotal	7	5/7	7
Localized pulmonary disease	7	0/7	4
Unknown history[c]	4		
Total	18	5/14	11

[a] Clarithromycin monotherapy was given for a portion or all of the patients' therapy.
[b] Recovery of the organism from blood or from multiple noncontiguous sites.
[c] One specimen was from skin, one was from urine, and two were from blood.

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TABLE 3. Clarithromycin MICs and sequencing results for the peptidyltransferase region of the 23S rRNA gene in isolates of *M. chelonae* and *M. abscessus* which developed resistance to clarithromycin

Organism and strain	Isolate	Date of isolation (mo/yr)	Source	Clarithromycin MIC (μg/ml)	23S peptidyltransferase region
M. chelonae					
MC963	Pre-Rx[a]	11/1991	Leg	0.125	Wild type
	Relapse	5/1992	Ankle	>16	A2059→G
MC1210	Pre-Rx	3/1993	Arm	0.125	Wild type
	Relapse	5/1993	Arm	>128	A2058→G
	Relapse	10/1993	Leg	32	A2058→G
MC1062	Relapse	5/1992	Sputum	>16	A2058→G
MC1575	Relapse	3/1995	Skin	16	A2059→G
MC1438	Pre-Rx	7/1994	Femur	0.125	—[b]
	Relapse	8/1994	Leg	>128	A2058→C
MC1590		5/1995	Urine	64	—
M. abscessus					
MC879	Pre-Rx	6/1991	Sputum	0.25	Wild type
	Relapse	8/1992	Sputum	>32	A2059→C
	Relapse	9/1993	Sputum	64	A2059→C
MC958	Pre-Rx	11/1991	Blood, skin, BAL[c]	≤0.063	Wild type
	Relapse	3/1992	Sputum	>16	A2059→G
	Relapse	3/1992	Urine	0.25[d]	A2059→G
	Relapse	6/1992	Thigh	1	—
	Relapse	9/1992	BAL	>32	A2059→G
MC1082	Pre-Rx	8/1992	Lung	0.25	Wild type
	Relapse	11/1993	CSF[e]	>128	A2059→C
	Relapse	10/1993	Stool	>128	A2059→C
MC1245	Pre-Rx	4/1992	Sputum	≤0.032	—
	Relapse	5/1993	Sputum	>128	A2058→G
MC1448	Relapse	8/1994	Sputum	>128	A2058→G
	Relapse	8/1994	Chest wound	>128	A2058→G
MC1549	Pre-Rx	10/1994	Skin	Susceptible[f]	—
	Relapse	3/1995	Sternum	64	A2059→G
	Relapse	5/1995	BAL	64	A2059→G
MC1554	—	3/1995	Blood	>64	A2059→G
MC1568	Relapse	5/1995	Sputum	>64	A2059→G
MC1081	Pre-Rx	1992	Sputum	0.25	—
	Relapse	10/1995	Sputum	>64	—
MC1561	Pre-Rx	4/1995	Sputum	0.063	—
	Relapse	6/1995	Sputum	>64	—
MC1599	Relapse	6/1995	Blood	>64	—
MC1636	—	5/1995	Blood	>64	—

[a] Pre-Rx, pretreatment.
[b] —, not done or not available.
[c] BAL, bronchoalveolar lavage.
[d] Single colonies picked from the plates revealed both susceptible and resistant colonies.
[e] CSF, cerebrospinal fluid.
[f] MIC not determined.

isolates were analyzed for their DNA fingerprint patterns. This included the isolates for which MICs were characterized (Table 1). Seven isolates of *M. chelonae* were obtained from three patients, and 18 isolates of *M. abscessus* were obtained from the remaining six patients. Isolates from three of the six patients infected with *M. abscessus* yielded only broken genomic DNA, as determined by PFGE, and could not be assessed. The remaining isolates from six patients gave intact genomic DNA and were evaluated for their *Dra*I and *Xba*I digestion patterns. All isolates with intact DNA from the same patient produced

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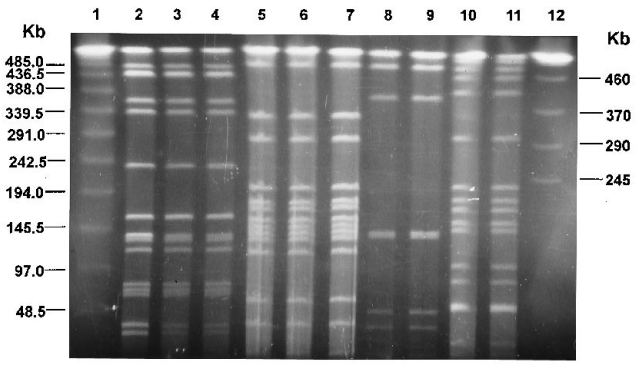

FIG. 1. PFGE of large restriction fragments of genomic DNA digested with *Dra*I. Included are pretreatment isolates (first isolate of each group) and relapse isolates (all subsequent isolates listed as isolate #2 or #3 in each group) from one patient with *M. abscessus* infection (lanes 2 to 4) and three patients with *M. chelonae* infection (lanes 5 to 7, 8 and 9, and 10 and 11, respectively) treated with clarithromycin. Lane 1, linear DNA standards; lane 2, *M. abscessus* MC1082; lane 3, *M. abscessus* MC1082#2; lane 4, *M. abscessus* MC1082#3; lane 5, *M. chelonae* MC1210; lane 6, *M. chelonae* MC1210#2; lane 7, *M. chelonae* MC1210#3; lane 8, *M. chelonae* MC1438; lane 9, *M. chelonae* MC1438#2; lane 10, *M. chelonae* MC963; lane 11, *M. chelonae* MC963#2; lane 12, yeast DNA linear standards.

identical *Dra*I and *Xba*I patterns (Fig. 1). For the nine isolates from three patients whose DNA was broken or lysed during processing and which could not be assessed by PFGE (37), good PCR patterns were obtained by random amplified polymorphic DNA PCR. As with PFGE, isolates from the same patient gave identical patterns, while isolates from different patients gave different patterns.

Southern blot analysis. Genomic DNAs from *M. smegmatis*, *M. bovis* BCG, *M. parafortuitum*, *M. senegalense*, *M. chelonae*, *M. abscessus*, *M. avium*, *M. terrae*, and *M. nonchromogenicum* isolates were digested with *Bam*HI and were hybridized to a 16S rRNA probe. This probe spans from positions 9 to 802 of the rRNA gene (*Escherichia coli* nomenclature), and none of the species investigated has an internal *Bam*HI site within the probe sequence according to nucleic acid sequence analyses. As can be seen in Fig. 2, *M. bovis* BCG, *M. avium*, and *M. abscessus* isolates showed single chromosomal 16S rRNA genes, whereas the other five species on the gel, i.e., *M. smegmatis*, *M. parafortuitum*, *M. senegalense*, *M. terrae*, and *M. nonchromogenicum*, harbored two chromosomal rRNA operons (the results for *M. chelonae* are not shown but are identical to those for *M. abscessus*).

DISCUSSION

The present study demonstrated that two species of rapidly growing mycobacteria, specifically, *M. chelonae* and *M. abscessus*, develop clinical resistance after exposure to clarithromycin by the same mechanism previously shown to be responsible for resistance in members of the *M. avium* complex (20, 21). That is, point mutations occur in the peptidyltransferase region of the 23S rRNA gene, with base pair substitutions for the adenine at position 2058 or 2059. Because in vitro mutations at these sites in *M. avium*, *M. intracellulare*, and *Streptomyces* spp. have also been associated with macrolide resistance (21, 26) and because position 2058 in the corresponding 23S rRNA is the usual site of dimethylation with erythromycin ribosomal methylase (*erm*) genes that confer macrolide resistance in most gram-positive bacteria, it is a reasonable assumption that these mutations are responsible for the development of macrolide resistance.

This finding was rather unexpected for two reasons. First, *M. chelonae* and *M. abscessus* isolates are known to harbor plasmids (34) and a number of heavy metal or antibiotic-modifying enzymes, including mercuric reductase (28), aminoglycoside acetyltransferase (34), TetK/L (25), and Ort A, B, and C (25). However, these antibiotic-modifying enzymes have not yet been shown to occur on plasmids, and the transfer of resistance by conjugation has thus far been unsuccessful in the rapidly growing mycobacteria. The second reason is that rapidly growing mycobacteria are believed to possess two rRNA operons (1, 24), which would likely necessitate two mutations to produce a resistance phenotype. However, Southern blot analysis revealed that *M. chelonae* and *M. abscessus* isolates, unlike other rapidly growing species such as *M. fortuitum*, *M. senegalense*, and *M. smegmatis*, have only a single rRNA operon (Fig. 2). Thus, in contrast to what was postulated on the basis of previous studies (1), the division of mycobacteria into rapidly and slowly growing organisms does not strictly correlate with the number of rRNA operons, i.e., slow growers harboring a single rRNA operon and rapid growers having two rRNA operons. In addition, two species of slow growers not previously studied, i.e., *M. terrae* and *M. nonchromogenicum*, were found to have two genomic rRNA operons (Fig. 2).

There are some differences between the current results and the in vivo mutations observed among *M. avium* isolates from AIDS patients with disseminated disease, although smaller numbers of isolates were studied in the present study. In two recently completed studies of mutational resistance among 46 such isolates of *M. avium* (21, 24), more than 90% of mutations involved A2058. In the current study, only 38% involved A2058 but 62% involved A2059. A recent study with *Helicobacter pylori* showed that the majority of mutational changes in that species also involved A2059 (32). In addition, multiple mutations in *M. avium* isolates from the same patient were identified among 60% of the clarithromycin-resistant isolates from the blood of patients with AIDS (21). Most commonly, this involved the presence of organisms with either A2058 → C or A2058 → G in the same blood culture or in different blood cultures for the same patient. Among the 20 relapse isolates

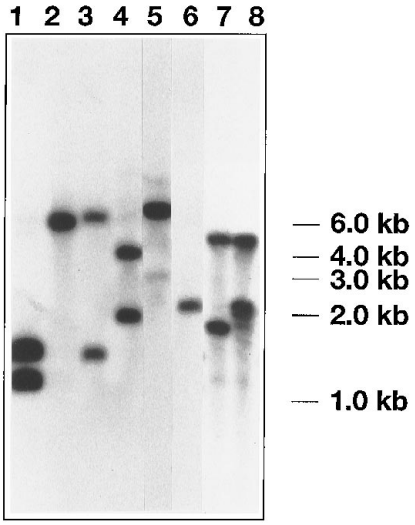

FIG. 2. Southern blot analysis to determine the number of rRNA operons. Genomic DNA (200 ng) was prepared, digested to completion with *Bam*HI, and hybridized with a 16S rRNA gene probe under stringent conditions. The size of the fragment was determined by comparison with the 1-kb ladder (Gibco BRL). Lane 1, *M. smegmatis*; lane 2, *M. bovis* BCG; lane 3, *M. parafortuitum*; lane 4, *M. senegalense*; lane 5, *M. avium*; lane 6, *M. abscessus*; lane 7, *M. terrae*; lane 8, *M. nonchromogenicum.*

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from 13 patients evaluated in the current study, only a single mutation was evident per patient, even though 6 of 11 patients for whom histories were available had disseminated disease. Either the numbers of organisms or the type of disease, or both, in AIDS patients with disseminated *M. avium* disease (but not *M. abscessus* or *M. chelonae* disease in patients without AIDS) is conducive to the development of a population of organisms with mixed mutations.

The present study also validates the suggested breakpoint for resistance to clarithromycin, ≥16 μg/ml when tested in Mueller-Hinton broth (14). Among isolates of *M. chelonae* and *M. abscessus*, 100% of isolates for which the MICs were in this range had a 23S ribosomal gene point mutation, while for all untreated wild-type strains clarithromycin MICs were ≤4 μg/ml (5).

Of the 800 isolates of the two species (one isolate per patient) tested for clarithromycin susceptibility, only 18 were found to be clarithromycin resistant. Because *M. chelonae* and *M. abscessus* isolates are generally susceptible only to the macrolides among the oral antimicrobial agents, it is assumed that one of these drugs was commonly used for therapy, although data to confirm this supposition are not readily available. This would suggest that the overall incidence of acquired clarithromycin resistance was quite low. The clinical disease represented among the nine patients for whom histories were available and whose isolates acquired clarithromycin resistance was quite limited. That is, all patients had either pulmonary or disseminated disease. The most common disease among patients infected with rapidly growing mycobacteria, posttraumatic wound infections (35), was not present among the patients in the present study. This suggests that clarithromycin monotherapy in nonimmunosuppressed hosts with localized wound infections caused by *M. chelonae* or *M. abscessus* isolates only rarely results in acquired resistance and is generally acceptable. A previous monotherapy trial of clarithromycin for cutaneous disease caused by *M. chelonae* in immunosuppressed patients (all patients were on corticosteroids) resulted in acquired resistance among isolates from 1 of 10 (10%) patients with disseminated disease and none of 4 (0%) patients with localized disease (36). No data on the relative incidence of mutational resistance in isolates from patients with disseminated disease caused by *M. abscessus* are available. In patients with lung disease caused by *M. abscessus* isolates the authors' clinical impression is that the risk of clarithromycin resistance with monotherapy is low (<10%). Because of the large number of organisms often present in patients with lung or disseminated disease caused by *M. chelonae* or *M. abscessus* isolates, combination therapy should be given when possible, at least initially, to try to prevent mutational resistance. This might include amikacin, cefoxitin, or clofazimine for *M. abscessus* infections and tobramycin, imipenem, or clofazimine for *M. chelonae* infections.

Despite the uniform susceptibilities of untreated strains of *M. chelonae* and *M. abscessus* to clarithromycin, not all rapidly growing mycobacteria have this pattern. Previous studies have shown that approximately 20% of isolates of *M. fortuitum*, most sorbitol-positive isolates of the *M. fortuitum* third biovariant complex (5), and *M. smegmatis* (32a) are resistant to 4 μg of clarithromycin per ml, and for most of these isolates, MICs are ≥32 μg/ml. The mechanism for this resistance has not been determined, but it may relate to a transferable resistance factor such as an erythromycin ribosomal methylase (*erm*) gene rather than a ribosomal mutation. Variable resistance to the tetracyclines also exists in these species, and a 1994 study demonstrated that some resistant strains carry recognized resistance determinants such as TetK/L and OrtABC (25). These resistant strains remain susceptible to the glycylcyclines, a new class of tetracyclines which are unaffected by the two major genetic mechanisms responsible for bacterial tetracycline resistance (4). Other recognized resistance determinants in these species include aminoglycoside acetyltransferase (34) and mercury-reducing enzymes (28). An erythromycin resistance factor must have a limited host range, because most species (complexes) of rapidly growing mycobacteria tested to date (i.e., *M. chelonae*, *M. abscessus*, *M. peregrinum*, and sorbitol-negative *M. fortuitum* third biovariant complex) are uniformly susceptible to clarithromycin (MICs, ≤4 μg/ml) (5, 32a). Thus, although all resistance to clarithromycin identified in *M. chelonae* and *M. abscessus* isolates in the present study was a result of ribosomal mutations, such may not be the case for other species of rapidly growing mycobacteria such as *M. fortuitum.*

Resistance to macrolides acquired by mutation generally does not occur with any recognizable incidence among bacterial species (with the exception of *H. pylori* isolates) (31). This is believed to relate to the presence of multiple copies of the ribosomal operon within the genome, such that a mutation involving the macrolide binding site of only one of the 23S rRNA gene copies does not affect the capability of macrolides to block essential transcriptional events. *H. pylori* isolates differ from most other bacterial species in that they contain only two copies of the ribosomal operon, and clarithromycin resistance related to mutational changes in the 23S ribosome occurs commonly (32). Studies of *M. avium* complex organisms (20, 21, 24) and now *M. chelonae* and *M. abscessus* isolates have shown that their genomes contain only a single copy of the ribosomal operon, hence their susceptibility to a single point mutation. *M. fortuitum* isolates contain two copies of the ribosomal operon, and to date we have failed to identify mutational resistance in any of these isolates (34a). The relationship of the number of copies of the ribosomal operon and the risk of phenotypic (clinical) resistance to clarithromycin following single point mutations have not been established. The presence of multiple copies does not eliminate the risk of expression or dominance of the resistance operon, with the resultant phenotypic resistance, a point clearly evident from clinical trials with clarithromycin for *H. pylori* (32), which has two copies of the rRNA operon, and from mutational studies of *Streptomyces ambofaciens* (26), which has four copies of the rRNA operon.

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