ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2000, p. 3241–3242 Vol. 44, No. 11
0066-4804/00/$04.00+0

Detection and Characterization of a Macrolide 2′-Phosphotransferase from a *Pseudomonas aeruginosa* Clinical Isolate

Macrolides, such as erythromycin, clarithromycin, and roxithromycin, have been used as long-term chemotherapy for diffuse *Pseudomonas aeruginosa* panbronchiolitis in Japan (2). In the United Kingdom, azithromycin has been used for cystic fibrosis patients infected with *P. aeruginosa* (3). Recently, highly macrolide-resistant strains, producing erythromycin esterase (1, 6) or macrolide 2′-phosphotransferase [MPH(2′)] (5, 7, 9), have been recovered with increasing frequency in clinical isolates of members of the family *Enterobacteriaceae* and also staphylococci (8). No reports, however, have yet been published regarding the presence of a macrolide-inactivating enzyme in *P. aeruginosa*. The appearance of enzymatically mediated high-level macrolide resistance among recent isolates of *P. aeruginosa* and the genotypes were investigated in this study.

A total of 287 clinical isolates were collected as one sample per patient in hospitals across Japan from 1996 to 1998. The MICs of macrolides were determined by the agar dilution method (4). The MICs of erythromycin at which 50 and 90% of the isolates were inhibited were 200 and 400 μg/ml, respectively. MICs of various macrolides for two isolates, M397 and M398, highly resistant to erythromycin are shown in Table 1. These isolates were highly resistant to all 14- and 15-membered-ring macrolides, whereas the MICs of 16-membered ring macrolides for them were almost similar to the corresponding MICs for the macrolide-susceptible strain PAO2142Rp. The two isolates showed similar patterns of multiple drug resistance including carbenicillin, tetracycline, chloramphenicol, streptomycin, and kanamycin. Transfer of the macrolide resistance phenotype from these *P. aeruginosa* isolates could not be demonstrated, as the transfer frequencies to the recipient strain *P. aeruginosa* PAO2142Rp were less than 10^{-8}.

Enzymatic inactivation of macrolides using crude extracts with or without a cofactor (40 mM ATP, 40 mM GTP, 2 mM acetyl coenzyme A, 40 mM NAD, 40 mM NADP, 40 mM UDPG, or 80 mM GSH) was determined by measuring residual macrolide activity (6). It was demonstrated that the inactivation of oleandomycin using crude extracts from the two isolates was dependent on only ATP or GTP (Table 1). R_f values of inactivated oleandomycin produced by the crude extracts were in agreement with that of standard oleandomycin 2′-phosphate by thin-layer chromatography (5). This suggested that isolates M397 and M398 produced an MPH(2′) enzyme. The substrate specificities of the enzyme activity with ATP using crude extract from isolate M398 for oleandomycin, triacetyloleandomycin, erythromycin, clarithromycin, roxithromycin, azithromycin, josamycin, leucomycin, midecamycin, miokamycin, spiramycin, and tylosin were 100, 100, 88, 73, 44, 15, <2, <2, <2, <2, <2, and <2%, respectively.

Primers for the macrolide-resistance genes *ereA*, *ereB*, *ermA*, *ermB*, *ermC*, *mphA* (8), and *mphB* (6) were used to generate specific PCR products. PCR products obtained with the *mphA* primers were detected for both isolates, M397 and M398 (Table 1). However, the identity of the DNA base sequence between the PCR product from isolate M398 and the *mphA* gene was 53%. These results suggest that a new *mph* gene has been detected in *P. aeruginosa* (accession no. AB048591).

The appearance of MPH(2′)-producing *P. aeruginosa* may be a warning to avoid the abuse of macrolide antibiotics and a caution for the future use of macrolides in long-term chemotherapy.

This study was supported by a grant from the Ministry of Health and Welfare, Japan, 2000, for molecular characterization of antibiotic resistance and development of methods for rapid detection of drug-resistant bacteria.

REFERENCES

1. **Arthur, M., A. Andremont, and P. Courvalin.** 1987. Distribution of erythromycin esterase and rRNA methylase genes in members of the family *Enterobacteriaceae* highly resistant to erythromycin. Antimicrob. Agents Chemother. **31:**404–409.
2. **Homma, H., A. Yamanaka, S. Tanimoto, M. Tamura, Y. Chijimatsu, S. Kira, and T. Izumi.** 1983. Diffuse panbronchiolitis: a disease of the transitional zone of the lung. Chest **83:**63–69.
3. **Jaffe, A., J. Francis, M. Rosenthal, and A. Bush.** 1999. Long-term azithromycin may improve lung function in children with cystic fibrosis. Lancet **351:** 420.
4. **Japan Society of Chemotherapy.** 1981. Method of MIC determination. Chemotherapy **29:**76–79.
5. **Kono, M., K. O'Hara, and T. Ebisu.** 1992. Purification and characterization of macrolide 2′-phosphotransferase type II from a strain of *Escherichia coli* highly resistant to macrolide antibiotics. FEMS Microbiol. Lett. **97:**89–94.
6. **Nakamura, A., K. Nakazawa, I. Miyakozawa, S. Mizukoshi, K. Turubuchi, M. Nakagawa, K. O'Hara, and T. Sawai.** 2000. Macrolide esterase-producing *Escherichia coli* clinically isolated in Japan. J. Antibiot. **53:**516–524.
7. **O'Hara, K., T. Kanda, K. Ohmiya, T. Ebisu, and M. Kono.** 1989. Purification

TABLE 1. MICs of macrolides against *P. aeruginosa* producing a macrolide-inactivating enzyme and properties of the enzyme

Strain	MIC (μg/ml)[a]													Inactivation of oleandomycin by crude enzyme with the cofactor:			*mphA* primer PCR products
	14-MR[b]					AZM (15-MR)	16-MR										
	OL	TAO	EM	CAM	RXM		JM	LM	MDM	MOM	RKM	SPM	TYL	ATP	GTP	Other	
M397	>1,600	>800	>1,600	>800	>800	>1,600	>400	>800	>200	>200	>400	>1,600	800	+	+	−	+
M398	>1,600	>800	>1,600	>800	>800	>1,600	>400	>800	>200	>200	>400	>1,600	800	+	+	−	+
PAO2142Rp[c]	>1,600	800	100	100	200	25	>400	800	>200	>200	400	1,600	800				

[a] OL, oleandomycin; TAO, triacetyloleandomycin; EM, erythromycin; CAM, clarithromycin; RXM, roxithromycin; AZM, azithromycin; JM, josamycin; LM, leucomycin; MDM, midecamycin; MOM, miokamycin; RKM, rokitamycin; SPM, spiramycin; TYL, tylosin.
[b] 14-MR, 14-membered ring.
[c] Recipient strain.

and characterization of macrolide 2′-phosphotransferase from a strain of *Escherichia coli* that is highly resistant to erythromycin. Antimicrob. Agents Chemother. **33:**1354–1357.
8. **Sutcliffe, J., T. Grebe, A. T. Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
9. **Taniguchi, K., A. Nakamura, K. Tsurubuchi, A. Ishii, K. O'Hara, and T. Sawai.** 1999. Identification of functional amino acids in the macrolide 2′-phosphotransferase II. Antimicrob. Agents Chemother. **43:**2063–2065.

Akio Nakamura
Izumi Miyakozawa
Kyoko Nakazawa
Koji O'Hara*
Tetsuo Sawai

Division of Microbial Chemistry
Faculty of Pharmaceutical Sciences
Chiba University, 1-33, Yayoi-cho, Inage-ku
Chiba 263-8522, Japan

*Phone: 81-3-3260-6725, ext. 5069
E-mail: oharak@ps.kagu.sut.ac.jp