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Macrolide Resistance in *Streptococcus pneumoniae* in Hong Kong

MARGARET IP,[1]* DONALD J. LYON,[1] RAYMOND W. H. YUNG,[2] COLIN CHAN,[1] AND AUGUSTINE F. B. CHENG[1]

Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin,[1] and Pamela Youde Nethersole Eastern Hospital,[2] Hong Kong, China

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Erythromycin resistance rates among penicillin-susceptible *Streptococcus pneumoniae* were 38 and 92% among penicillin-intermediate and -resistant *S. pneumoniae* isolates from Hong Kong, respectively, and 27% (43 of 158) of the isolates showed the MLS_B phenotype, and the majority carried the *ermB* gene; 73% (115 of 158) displayed the M phenotype, and all possessed the *mef* gene. The MLS_B phenotype was predominant in penicillin-susceptible, macrolide-resistant isolates and in penicillin-nonsusceptible isolates of serotype 6B, whilst the M phenotype was predominant in penicillin-intermediate or -resistant isolates belonging to serotype 23F or 19F. Extensive spread of clones of drug-resistant pneumococci has led to the widespread presence of macrolide resistance in *S. pneumoniae* in Hong Kong.

Macrolide resistance among *Streptococcus pneumoniae* has increased in many countries, such as the United States (2, 3) and United Kingdom (4). Two principal mechanisms of macrolide resistance have been described (10); target modification is mediated by an rRNA erythromycin resistance methylase and coded by the *ermB* (*ermAM*) gene. These organisms express the MLS_B phenotype and show broad cross-resistance to macrolides, lincosamides, and streptogramin B antibiotics. The M phenotype involves an active efflux pump, which removes only 14-membered and 15-membered macrolides from the bacterial cell (14). The determinant was identified to be the *mef* (*mefE*) gene (15). Isolates showing the M phenotype are susceptible to clindamycin and streptogramin B. In 1999, two further mechanisms were described, one mediated by alterations in the L4 ribosomal protein, and another due to mutation in the 23S rRNA (15a). The incidence of erythromycin resistance in pneumococci at the Prince of Wales Hospital, Hong Kong, has risen from 22% in 1993 to 42% in 1997 (6). The likely explanation is the dissemination of a few clones of multidrug-resistant *S. pneumoniae* in the Hong Kong population as previously described (5). The objective of the present study was to investigate the prevalence and distribution of the macrolide resistance determinants in isolates of *S. pneumoniae* in Hong Kong. In addition, the relationships between these determinants, penicillin susceptibility, and their serotypes were also determined.

A total of 197 strains of *S. pneumoniae* isolated at the Prince of Wales Hospital and Pamela Youde Nethersole Eastern Hospital, Hong Kong, from 1994 to 1998 were studied. Approximately 25% were invasive isolates from blood and cerebrospinal fluid, and the remainder were from sputum which had reduced penicillin susceptibility by the 1-μg oxacillin disk method. The two hospitals are located in the northern and southern parts of Hong Kong, and the isolates were thus deemed to be representative of strains from patients with pneumococcal infections requiring hospital admissions in Hong Kong. The penicillin-intermediate or -resistant isolates had previously been well characterized by molecular typing using *pbp* gene profiles and pulsed-field gel electrophoresis (PFGE) (5).

MICs of penicillin, erythromycin, clarithromycin, and clindamycin were determined by the agar dilution method (8). Inocula of 10^4 CFU/spot were incubated at 35°C for 18 h on Mueller-Hinton agar supplemented with 5% defibrinated horse blood. *S. pneumoniae* ATCC 6315 and 49619 were included as controls. Inducible resistance to clindamycin was detected by the method previously described (11). Conserved primer sets were chosen to amplify a 640-bp fragment of the *erm* methylase genes as follows: *ermA*, 5′-TCTAAAAAGCATGTAAAAGAA-3′ and 5′-CTTCGATAGTTTATTAATATTAGT-3′; *ermB*, 5′-GAAAAGGGTACTCAACCAAATA-3′ and 5′-AGTAACGGTACTTAAATTGTTTAC-3′; and *ermC*, 5′-TCAAAACATAATATAGATAAA-3′ and 5′-GCTAATATTGTTTAAATCGTCAAT-3′. A 1.2-kb fragment of the *mef* gene in *S. pneumoniae* was amplified using primer pair 5′-GAAAAATACAACAATTGGAAAC-3′ and 5′-AATCTAATTTTCTAACCTCA-3′. PCRs were performed on the OmniGene DNA thermal cycler (Hybaid) using an initial denaturation at 94°C for 5 min followed by 35 cycles of amplification at 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s. A final elongation step was performed at 72°C for a further 7 min. All the strains were serotyped by the chessboard agglutination or the quellung reaction method using Pneumotest antisera (Statens Seruminstitut, Copenhagen, Denmark).

A total of 80% (158 of 197) of the *S. pneumoniae* strains were resistant to erythromycin with a MIC of ≥1.0 μg/ml, 27% (43 of 158) showed an MLS_B phenotype with erythromycin and clindamycin 50% and 90% MICs (MIC_{50} and MIC_{90}) of >64 μg/ml; 73% (115 of 158) showed resistance to erythromycin alone, with MIC_{50} and MIC_{90} of 8.0 μg/ml. The MICs of the antibiotics tested for these two groups are shown in Table 1. Of

* Corresponding author. Mailing address: Dept. of Microbiology, Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, China. Phone: (852) 2632 2306. Fax: (852) 2647 3227. E-mail: margaretip@cuhk.edu.hk.

TABLE 1. MICs of 197 Hong Kong *S. pneumoniae* isolates for four antibiotics by macrolide and lincosamide susceptibility phenotypes

Macrolide phenotype[a] (no. of strains)	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Penicillin			
MLS_B (43)	<0.03–2.0	1.0	2.0
M (115)	<0.03–4.0	1.0	2.0
S (39)	<0.03–2.0	0.03	1.0
Erythromycin			
MLS_B (43)	8.0–>64.0	>64.0	>64.0
M (115)	1.0–64.0	8.0	8.0
S (39)	0.12–0.25	0.25	0.25
Clarithromycin			
MLS_B (43)	8.0–>64.0	>64.0	>64.0
M (115)	1.0–64.0	8.0	8.0
S (39)	0.12–0.25	0.25	0.25
Clindamycin			
MLS_B (43)	1.0–>64.0	>64.0	>64.0
M (115)	0.12–0.5	0.25	0.25
S (39)	0.12–0.25	0.25	0.25

[a] MLS_B, macrolide and lincosamide resistance phenotype; M, erythromycin-resistant, clindamycin-susceptible phenotype; S, erythromycin- and clindamycin-susceptible isolates.

TABLE 2. Serotypes of *S. pneumoniae* in relation to penicillin and macrolide susceptibility[a] (197 isolates)

Serotype and macrolide phenotype	No. of isolates with the following penicillin susceptibility:	
	S	I or R
23F		
S	3	9
R_{MLSB}	3	5
R_M	2	64
19F		
S	1	0
R_{MLSB}	0	3
R_M	0	39
6B		
S	1	3
R_{MLSB}	0	23
R_M	2	3
14		
S	0	0
R_{MLSB}	3	2
R_M	2	3
Other		
S	21	1
R_{MLSB}	4	0
R_M	0	0
Total	42	155

[a] S, sensitive; I, intermediate; R, resistant; MLSB, macrolide and lincosamide resistance phenotype; M, erythromycin-resistant, clindamycin-susceptible phenotype.

the isolates expressing the MLS_B phenotype, only the *ermB* gene was detected in 93% of the isolates by PCR. Three isolates were repeatedly negative on testing for *ermA, -B,* or *-C*, and hence perhaps other mechanisms of macrolide resistance exist. All the isolates expressing the M phenotype were positive for the *mef* gene by PCR. Table 2 shows the serotypes of *S. pneumoniae* in relation to penicillin and macrolide susceptibility. Among the 42 penicillin-susceptible isolates, 16 (38%) isolates were resistant to erythromycin with a MIC of ≥1.0 μg/ml, and 62.5% of the penicillin-susceptible, macrolide-resistant isolates showed the MLS_B phenotype. However, of the 155 penicillin-intermediate or -resistant isolates examined, 142 (92%) isolates were resistant to erythromycin, 107 of 142 (75%) of these belonged to the M phenotype, whilst the remainder (35 of 142, 25%) belonged to the MLS_B phenotype, with a MLS_B/M phenotype ratio of 1:3. The MLS_B phenotype was mainly associated with serotype 6B ($P > 0.001$, chi square test), whilst the M phenotype was predominant in isolates expressing serotype 23F or 19F ($P > 0.001$, chi square test).

The study indicated a high percentage of erythromycin resistance among clinical isolates of *S. pneumoniae* in Hong Kong, with 38% in penicillin-susceptible pneumococci and 92% of penicillin-intermediate or -resistant isolates. Our previous study (5) showed that these strains belonging to 19F and 23F had identical *pbp* profiles and PFGE fingerprints and belong to clones indistinguishable from that belonging to the Spanish 23F clone. Similarly, isolates belonging to serotype 6B had unique *pbp* profiles and identical PFGE fingerprints to that of the Spanish 6B clone (5). The data further support the hypothesis that the dissemination of particular clones of *S. pneumoniae* of reduced penicillin susceptibility has been responsible for the spread of macrolide resistance determinants in Hong Kong. In Canada and the United States, the M phenotype was prevalent in 55.8 and 71%, respectively, of the macrolide-resistant pneumococci (3, 7). In contrast, the MLS_B resistance mechanism predominates in European countries, and the *mef* gene was only found in 1, 5.8, and 9% among resistant pneumococci in France (P. Angot, M. Vergnaud, and R. Leclerq, Program Abstr. 39th Intersci. Conf. Antimicrob. Agents. Chemother. abstr. 1221, 1999), Italy (9), and Belgium (1) respectively. Macrolide resistance has been reported to be high among pneumococci in Asian countries (13), but the distribution of these macrolide resistance determinants is not known. As in Hong Kong, penicillin- and multidrug-resistant *S. pneumoniae* clones belonging to serotypes 19 and 23 disseminated widely in the last decade in Taiwan (12), Korea (17), and Japan (18), and it is likely that the *mef* genes are prevalent among the penicillin-resistant pneumococci in these countries too.

The 14- and 15-membered macrolides have poor activity against Hong Kong clinical isolates of *S. pneumoniae*, particularly against penicillin-intermediate or -resistant strains. This study supported the widespread dissemination of the macrolide resistance determinants, particularly the *mef* gene, which occurred with the spread of penicillin-nonsusceptible pneumococcal clones in Hong Kong.

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