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Erythromycin-Resistant Group A Streptococcal Isolates Collected between 2000 and 2005 in Oahu, Hawaii, and Their *emm* Types

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We examined erythromycin and clindamycin susceptibilities with Etest methodology among 546 group A streptococcal isolates collected in Hawaii between February 2000 and November 2004. Erythromycin resistance was low (3.1%). No isolate was clindamycin resistant. The prevalence of erythromycin resistance in group A streptococci remains low in Hawaii.

The epidemiology of group A streptococcal (GAS) infections in Hawaii is unusual for the United States, with no seasonal differences in pharyngitis and impetigo rates (2, 9, 24). More important, the incidence rates for sequelae of GAS infections, such as acute rheumatic fever, have remained high (incidence of 9.5 to 12.4/100,000 persons/year) among Hawaiian and Polynesian ethnic groups (14). This unique public health problem dictates appropriate treatment of the GAS infections. Since macrolides are not infrequently used to treat GAS infections, the purpose of this study was to determine the current erythromycin and clindamycin susceptibilities of GAS isolates from Hawaii and to determine whether resistance is associated with the prevalence of specific *emm* types (1).

(These data were presented in part at the annual meeting of the Pediatric Academic Societies in San Francisco, CA, in May 2004.)

A total of 546 GAS isolates collected in Hawaii between February 2000 and November 2004 was examined. With the exception of isolates collected in 2003, when 32 isolates were collected from healthy school children as part of a pharyngitis surveillance study, all strains were isolated from patients with clinical infections. Most isolates were from throat (440 isolates; 80.6%) and wound (89 isolates; 16.3%) cultures. Three cerebrospinal fluid, five deep tissue, eight blood, and one joint fluid isolate were included among the 546 strains studied. The numbers of isolates were 44, 81, 252, 32, and 137 for the years 2000, 2001, 2002, 2003, and 2004, respectively.

Etest methodology (AB Biodisk, Piscataway, NJ) was used to determine MICs for erythromycin and clindamycin according to the National Committee for Clinical Laboratory Standards (NCCLS) breakpoints (17, 18). The erythromycin-clindamycin double disk test was applied to resistant isolates (11) by using disks of erythromycin (15 μg) and clindamycin (2 μg) (Becton, Dickinson and Company, Sparks, MD) placed 15 mm apart. "Blunting" of the inhibition zone around the clindamycin disk proximal to the erythromycin disk was considered to be inducible resistance (7) (iMLS$_b$ phenotype), and susceptibility to clindamycin with no blunting indicated the M phenotype. PCR assays were used to identify the genetic mechanism of resistance of erythromycin-resistant GAS isolates. The *mef*(A), *erm*(B), *erm*(A), and *erm*(TR) resistance genes were detected by PCR amplification with primers described previously (5, 16, 19).

GAS isolates were characterized by *emm* sequencing as previously described (http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm). Thirty isolates were also characterized by M protein/T agglutination pattern analysis and by the serum opacity factor by previously described techniques (13).

Among the 546 isolates, only 17 (3.1%) were erythromycin resistant; 16 of them were from throat swabs of patients with pharyngitis, and 1 strain was from a patient with a wound infection (Table 1). The erythromycin MICs of the resistant strains were between 1 and 8 μg/ml. Twelve of the 17 resistant strains were isolated in 2002 (6.7% of the 2002 isolates). No changes in the population or rate of upper respiratory infections were noted in 2002. Two (12.5%) throat isolates which were resistant to erythromycin showed intermediate susceptibility to clindamycin. There were no isolates resistant to clindamycin.

Five of the 17 (29.4%) erythromycin-resistant isolates were identified as having the iMLS$_b$ phenotype. The iMLS$_b$ phenotype isolates had the *erm*(A) and *erm*(TR) genes [the latter is a variant of the *erm*(A) gene]. Eleven isolates had the M phenotype. One isolate was resistant to both antibiotics (cMLS$_b$ phenotype) and had the *erm*(B) gene.

Five hundred sixteen isolates were characterized by sequencing the *emm* gene. The remaining thirty isolates were characterized by M protein/T agglutination pattern analyses and by serum opacity factor studies. Among the total 546 isolates studied, 70 different *emm*/M types were found. The most common *emm* types were *emm* 12, 1, 28, 4, 92, 22, 85, 81, 58, 49,

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TABLE 1. Isolation years, sources, *emm* typing, MICs of erythromycin and clindamycin, phenotypes, and resistance genes identified in resistant GAS isolates

Isolate	Yr isolated	Source	*emm* type	MIC (μg/ml) for:		Phenotype	Resistance gene(s)
				Erythromycin	Clindamycin		
1	2001	Skin	114	3	0.94	$iMLS_b$	*erm*(A), *erm*(TR)
2	2001	Throat	58	2	0.125	$iMLS_b$	*erm*(A), *erm*(TR)
3	2002	Throat	1	1	0.19	M	*mef*(A)
4	2002	Throat	58	8	0.5	$iMLS_b$	*erm*(A), *erm*(TR)
5	2002	Throat	12	3	0.75	$cMLS_b$	*erm*(B)
6	2002	Throat	1	2	0.19	M	*mef*(A)
7	2002	Throat	94	6	0.25	$iMLS_b$	*erm*(A), *erm*(TR)
8	2002	Throat	28	3	0.19	M	*erm*(B)
9	2002	Throat	49	4	0.19	M	*mef*(A)
10	2002	Throat	101	1.5	0.25	M	*mef*(A)
11	2002	Throat	28	2	0.125	M	*mef*(A)
12	2002	Throat	89	4	0.125	M	*mef*(A)
13	2002	Throat	4	3	0.25	M	*mef*(A)
14	2002	Throat	69/65	3	0.19	M	*mef*(A)
15	2004	Throat	4	6	0.064	M	*me*(A)
16	2004	Throat	4	8	0.064	M	*erm*(A), *erm*(TR), *mef*(A)
17	2004	Throat	94	8	0.19	$iMLS_b$	*erm*(A), *erm*(TR)

69/65, 101, 75, 74, and 77. These *emm* types comprised 387 of 546 isolates (70.9%). *emm* types 1 (2 isolates), 4 (3 isolates), 12, 28 (2 isolates), 58 (2 isolates), 49, 69/65, 89, 94 (2 isolates), 101, and 114 were identified among the 17 erythromycin-resistant isolates.

Discussion. Widespread resistance to macrolides has been reported in several geographic areas (3-5, 10, 12, 15, 20, 23). Erythromycin resistance among the Hawaiian GAS isolates (3.1%) was lower than the 5.5% overall macrolide resistance rate found in the 2000-to-2001 PROTEKT US study and also lower than that found in a 2000-to-2003 U.S. surveillance study (8, 21). It is of interest that while Wittler et al. reported higher levels of resistance (9.5%) to erythromycin in a limited number of Hawaiian isolates a decade earlier (23), data from the present evaluation of a much larger number of GAS isolates indicated lower resistance rates in Hawaii. We are unable to compare present M protein types and sites of isolation with those reported in Wittler's earlier study.

Eleven different M protein-expressing types (*emm* 1, 4, 12, 28, 58, 49, 69/65, 89, 94, 101, and 114) were identified among the 17 erythromycin-resistant isolates. Several *emm* types, such as *emm* 1, 2, 4, 6, 8, 12, 28, 48, 58, 61, 75, 77, 89, 94, and 114, have been previously reported to have associations with erythromycin resistance (6, 7, 15, 21, 22). *emm* types 1, 4, 12, 28, 58, 89, 94, and 14 were also identified among our resistant isolates. The presence of the serum opacity factor gene has also been associated with erythromycin resistance (7). Interestingly, six of our resistant isolates belonged to five different *emm* sequence types known to lack this gene (http://www.cdc.gov/ncidod/biotech/strep.htm).

Knowledge of erythromycin resistance rates has considerable medical and public health significance in Hawaii, where the incidence rates of acute rheumatic fever and other sequelae remain high (2, 24). This is the first study done in Hawaii to determine whether erythromycin and clindamycin resistance has been associated with the prevalence of specific M protein types. Although uncommon *emm* types (when compared with isolates from the U.S. mainland) were identified, there was no preponderance of any specific *emm* type among the erythromycin-resistant GAS isolates. Overall, our data indicate that erythromycin resistance among GAS isolates has remained low in Hawaii through 2004 and that macrolides can be used in the treatment of GAS infections when indicated. However, high prevalence rates of nonsuppurative sequelae in Hawaii as well as rapid changes in macrolide resistance rates as observed in other communities in the United States and in other countries necessitate continuing vigilant surveillance for resistance.

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