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Prevalence and Mechanisms of Erythromycin Resistance in Group A and Group B *Streptococcus*: Implications for Reporting Susceptibility Results

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Increased rates of erythromycin resistance among group B *Streptococcus* (GBS) and group A *Streptococcus* (GAS) have been reported. Cross-resistance to clindamycin may be present, depending on the mechanism of resistance. We determined the prevalence of macrolide-resistant determinants in GBS and GAS isolates to guide the laboratory reporting of erythromycin and clindamycin susceptibility. Susceptibilities were determined by the disk diffusion and broth microdilution methods. Inducible and constitutive resistance to clindamycin was determined by the double-disk diffusion method. The presence of the *ermTR*, *ermB*, and *mefA* genes was confirmed by PCR. Of the 338 GBS isolates, 55 (17%) were resistant to erythromycin, whereas 26 (8%) were resistant to clindamycin. The *erm* methylase gene was identified in 48 isolates, 22 of which had inducible resistance to clindamycin and 26 of which had constitutive resistance to clindamycin. The remaining seven resistant isolates had *mefA*. Of the 593 GAS isolates, 49 (8%) and 6 (1%) isolates were resistant to erythromycin and clindamycin, respectively. Erythromycin resistance was due to *mefA* in 33 isolates, whereas 14 isolates had *erm*-mediated resistance (9 isolates had inducible resistance and 5 isolates had constitutive resistance). In our population, erythromycin resistance in GAS was predominantly mediated by *mefA* and erythromycin resistance in GBS was predominantly mediated by *erm*. Regional differences in mechanisms of resistance need to be taken into consideration when deciding whether to report clindamycin susceptibility results on the basis of in vitro test results. Testing by the double-disk diffusion method would be an approach that could be used to address this issue, especially for GAS.

In Canada, as in other regions of North America and Europe, the rates of erythromycin resistance among isolates of the group A *Streptococcus* (GAS; *Streptococcus pyogenes*) and the group B *Streptococcus* (GBS; *Streptococcus agalactiae*) have been increasing (1, 8, 16). In Ontario, Canada, the rate of erythromycin resistance among GBS isolates has increased from 5 to 13% over a period of 3 years (4). For GAS isolates it has increased from 2 to 14% over 4 years (12). In the United States, the rates of erythromycin resistance among GBS isolates increased from 12 to 20% between 1990 and 2000 (16). Despite these documented increases, there are geographic variations in resistance rates and the prevalence of resistance mechanisms (11). In one study, the rates of macrolide resistance among GAS isolates varied from 9% in large urban settings to 0% in rural areas, with an overall average of 4.6% (23).

Resistance to erythromycin in streptococci is mediated by two major mechanisms. Drug efflux, also referred to as the M phenotype, is encoded by the *mefA* gene and results in low-level resistance to erythromycin but not clindamycin. Resistance may also be due to methylation of the ribosomal drug binding site, which mediates resistance to macrolides, lincosamides, and streptogramin group B (MLS_B). Methylases are encoded by the *erm* genes and may be inducibly or constitutively expressed (13). Isolates with inducible MLS_B resistance test resistant to erythromycin and susceptible to clindamycin (19). In contrast, constitutive MLS_B resistance results in resistance to both erythromycin and clindamycin (19). At present, many laboratories report susceptibilities to erythromycin and clindamycin on the basis of in vitro test results without reference to the mechanisms of resistance. In this study, our goal was first to determine the prevalence of erythromycin and clindamycin resistance among clinical isolates of GBS and GAS from the Ottawa, Ontario, Canada, area. Second, we correlated the in vitro results with the mechanism of resistance to help guide the most appropriate approach to the reporting of clindamycin susceptibility.

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MATERIALS AND METHODS

Bacterial isolates. A total of 593 consecutive clinical isolates of GAS and 338 consecutive clinical isolates of GBS were collected from an adult hospital and a pediatric hospital in Ottawa from 2002 to 2003. Among the GAS isolates, 339 (57%) were recovered from pediatric specimens, and all were pharyngeal isolates. The remaining 254 (43%) GAS isolates were from specimens recovered from throats (51%), wounds (26%), blood and sterile sites (14%), and other sources (9%) from adults. The GBS isolates were recovered from vaginal-rectal swabs (32%), wounds (25%), urine (21%), blood and sterile sites (16%), and other sources (6%).

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TABLE 1. Comparison of phenotypes and genotypes of erythromycin-resistant GAS isolates[a]

Phenotype[b]	Genotype	No. of isolates	MIC (μg/ml)			
			Erythromycin		Clindamycin	
			90%	Range	90%	Range
M	*mefA*	33	32	8–32	0.06	0.06–0.125
$iMLS_B$	*ermB*	1		≥1,024		0.25
$iMLS_B$	*ermTR*	8	≥1,024	4–≥1024	0.25	0.06–0.5
$cMLS_B$	*ermB*	5	≥1,024	256–≥1024	512	1–512
Unknown		2		≥1,024		0.06–0.125

[a] A total of 49 isolates were tested.
[b] Abbreviations: M, efflux; $iMLS_B$, inducible MLS_B resistance; $cMLS_B$, constitutive MLS_B resistance.

Susceptibility testing. The MICs of erythromycin and clindamycin (Sigma Chemical Co., St. Louis, Mo.) for GAS, GBS, and appropriate quality control strains were determined by the broth microdilution method and were interpreted according to the recommendations of NCCLS (17, 18). Testing was performed with Mueller-Hinton broth supplemented with 5% lysed horse blood (Oxoid, Ottawa, Ontario, Canada).

Differentiation of macrolide resistance mechanisms by phenotypic characterization was performed by double-disk diffusion testing, as described previously (5, 9). Erythromycin (15 μg) and clindamycin (5 μg) disks (Oxoid) were placed 15 mm apart, edge to edge, on Mueller-Hinton agar supplemented with 5% sheep blood agar (Becton Dickinson Microbiology Systems, Sparks, Md.) that had been inoculated with a 0.5 McFarland suspension of the organism. The plates were incubated for 24 h at 35°C in 5% CO_2. Blunting was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disk, indicating MLS_B-inducible methylation. Resistance to both erythromycin and clindamycin indicated MLS_B-constitutive methylation. Resistance to erythromycin but susceptibility to clindamycin without blunting indicated an efflux mechanism (M phenotype).

Detection of erythromycin resistance genes. The *mefA*, *ermB*, and *ermTR* erythromycin resistance genes were detected by multiplex PCR with previously published sequences that were multiplexed with 16S rRNA gene-specific primers as an internal control (5, 14, 21). The methods used were adapted from a previous study (5), and PureTaq Ready-to-Go PCR beads (Amersham-Pharmacia Biotech) were used. Template DNA was prepared as described previously (5). Each 25-μl bead reaction mixture contained 5 μl of template, 0.25 μM each the *ermTR*- and *ermB*-specific primer sets, 0.062 μM the *mefA*-specific primer set, 0.13 μM the 16S rRNA gene-specific primer set, and $MgCl_2$ at a final concentration of 3.0 mM. The reactions were performed in a Perkin-Elmer 9600 thermocycler under the following conditions: denaturation at 95°C for 3 min and 35 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 1 min. A final elongation step was performed at 72°C for 5 min. The products were separated on a 2% agarose minigel with the expected sizes: *ermB*, 640 bp; *ermTR*, 400 bp; *mefA*, 348 bp; and 16S rRNA gene, 241 bp. Reactions were performed with the following controls: a triplex of a GAS-GBS template positive for *ermB*, *ermTR*, and *mefA*; a negative reagent control; and a negative antibiotic-susceptible GAS and GBS template. PCR detection of *linB* in erythromycin-susceptible, clindamycin-resistant GBS isolates was performed by the method and with the primers described previously (2); but for our use of the method, the 16S rRNA gene-specific primers were added to the reaction mixture, and the method was adapted for use with PureTaq Ready-to-Go PCR beads. The expected products were 925 bp (*linB*) and 241 bp (16S rRNA gene). Detection of the *ermA* gene (640 bp) in GAS isolates negative for *ermTR*, *ermB*, and *mefA* was similarly performed as described previously (21).

RESULTS

Erythromycin and clindamycin resistance in GAS isolates. Erythromycin resistance was detected in 49 (8%) of the 593 GAS isolates, and clindamycin resistance was detected in 6 (1%) of the 593 GAS isolates. Among the erythromycin-resistant isolates, 33 had the M phenotype, which was due to the presence of *mefA* in all 33 isolates; 5 isolates had constitutive MLS_B resistance due to the presence of *ermB*; and 9 isolates had inducible MLS_B resistance, which was detected in association with *ermB* in 1 isolate and in association with *ermTR* in the remaining 8 isolates. Two erythromycin-resistant isolates had undefined mechanisms of resistance (Table 1). The erythromycin and clindamycin MICs were consistent with the expected phenotypes. Isolates with *mefA*-mediated resistance had low-level resistance to erythromycin (MICs at which 90% of isolates are inhibited [MIC_{90}] = 32 μg/ml), and all isolates were susceptible to clindamycin (Table 1).

Erythromycin resistance in GAS isolates from adult and pediatric populations. Erythromycin resistance was found in 16 of 254 (6%) adult GAS isolates, whereas it was found in 33 of 339 (10%) pediatric GAS isolates (Table 2). Efflux encoded by *mefA* was identified in both pediatric and adult isolates but was more prevalent among pediatric isolates (72% of pediatric isolates versus 56% of adult isolates). Among the remaining resistant isolates, inducible MLS_B resistance (*ermTR*) was more prevalent among adult isolates (31%), whereas constitutive MLS_B resistance (*ermB*) was found equally among adult and pediatric isolates (13%) (Table 2).

Erythromycin and clindamycin resistance in GBS isolates. Among the 338 GBS isolates tested, 55 (17%) and 26 (8%) were resistant to erythromycin and clindamycin, respectively. Of the 55 erythromycin-resistant isolates, 7 displayed the M phenotype, which was due to *mefA* in all 7 isolates; 22 had an inducible MLS_B resistance phenotype, which was due to *ermTR* in all 22 isolates; and 26 had constitutive MLS_B resistance. Of the MLS_B-resistant isolates with the constitutive resistance phenotype, resistance was due to *ermB* in most isolates, but resistance was associated with *ermTR*, either alone or in combination with other mechanisms, in a small proportion

TABLE 2. Comparison of erythromycin resistance and associated mechanisms of resistance in adult isolates compared to those in pediatric isolates

Parameter	No. (%) of isolates	
	Adults (n = 254)	Pediatric population (n = 339)
Erythromycin resistance	16 (6)	33 (10)
Mechanism of resistance		
mefA	9 (56)	24 (72)
ermTR	5 (31)	3 (9)
ermB	2 (13)	4 (13)
Unknown		2 (6)

TABLE 3. Comparison of phenotypes and genotypes of erythromycin-resistant GBS isolates[a]

Phenotype[b]	Genotype	No. of isolates	MIC (μg/ml)			
			Erythromycin		Clindamycin	
			90%	Range	90%	Range
M	*mefA*	7	8	8–32	0.06	0.06–0.125
$iMLS_B$	*ermTR*	22	16	≥1,024	0.25	0.06–0.25
$cMLS_B$	*ermB*	19	≥1,024	4–≥1,024	1,024	4–≥1,024
$cMLS_B$	*ermTR*	5	8	256–≥1,024	≥1,024	512–≥1,024
$cMLS_B$	*ermTR*, *ermB*	1		≥1,024		≥1,024
$cMLS_B$	*ermTR*, *mefA*	1		≥1,024		≥1,024
L	*linB*	1		0.06		8
Unknown		2		0.06–0.125		0.5–2

[a] A total of 55 isolates were tested.
[b] Abbreviations: M, efflux; $iMLS_B$, inducible MLS_B resistance; $cMLS_B$, constitutive MLS_B resistance; L, lincomycin nucleotidyltransferase.

of the isolates (Table 3). Three additional isolates were found to be susceptible to erythromycin and resistant to clindamycin. Resistance was mediated by *linB* (L phenotype) in one isolate and was undefined in the remaining two isolates.

The distribution of clindamycin MICs for erythromycin-resistant isolates was consistent with the observed phenotype (Table 3). For isolates with the M and the inducible MLS_B resistance phenotypes, the clindamycin MIC remained below the NCCLS-defined breakpoint of 1 μg/ml (MIC_{90}s, 0.06 and 0.25 μg/ml, respectively). The MIC_{90}s of erythromycin for these isolates were above the breakpoints consistent with low-level resistance (8 and 16 μg/ml for the M and the inducible MLS_B resistance phenotypes, respectively) and were within 1 dilution of each other. Isolates with the constitutive MLS_B resistance phenotype were highly resistant to both erythromycin and clindamycin (Table 3).

DISCUSSION

For the reporting of clindamycin susceptibility, it is important to consider the significance of inducible methylation. Treatment failures with clindamycin have previously been reported for *Staphylococcus aureus* isolates with inducible MLS_B resistance encoded by *ermA* (7, 15, 20, 22). To address these concerns, NCCLS has revised its 2004 recommendations for testing and reporting of the clindamycin susceptibilities of staphylococci (17). Current recommendations are to test *S. aureus* and coagulase-negative *Staphylococcus* isolates for inducible MLS_B resistance by the double-disk diffusion test, and reports of clindamycin failure during therapy have been associated with this phenotype. Unlike *Staphylococcus* species, NCCLS has no recommendations for the routine testing of erythromycin-resistant GAS or GBS isolates for inducible MLS_B resistance. Concerns over the increasing incidence of macrolide resistance in GBS have recently prompted the Centers for Disease Control and Prevention to recommend routine erythromycin and clindamycin susceptibility testing in their guidelines for the prevention of perinatal GBS disease (3). However, inducible MLS_B resistance was not addressed. In the absence of direct evidence of the failure of clindamycin for the treatment of infections caused by streptococci with inducible methylation, the potential for a suboptimal outcome with clindamycin is suggested by the homology between the inducible *ermA* gene in *Staphylococcus* species and the inducible *ermTR* gene in GAS and GBS (13). Presumably, the failure of clindamycin treatment for infections caused by GAS and GBS isolates with inducible resistance may also be expected. Experimentally, the in vitro selection of *ermTR* GAS isolates with constitutive clindamycin resistance has been reported (10). The selection of constitutive expression was found to be due to alterations in the attenuator sequences of the *ermTR* gene in erythromycin-resistant isolates. Although we did not determine if similar alterations were present in our isolates, the fact that 20% of the clindamycin-resistant GBS isolates harbored the *ermTR* gene suggests that a high frequency of selection for constitutive resistance may also be expected for streptococci. These results are consistent with those of other studies that have found *ermTR* in a significant proportion of GBS isolates with constitutive resistance (4).

The implication for reporting of clindamycin resistance among GAS and GBS isolates will depend on the prevalence of erythromycin resistance and the mechanism of resistance. Assuming that inducible MLS_B resistance is clinically relevant, in our region, where the prevalence of the *erm* gene among erythromycin-resistant GBS isolates is approximately 90%, clindamycin susceptibility could be reported on the basis of in vitro test results or double-disk diffusion testing with erythromycin. Taking into consideration work flow issues and knowledge of local resistance trends, at the Division of Microbiology, Department of Laboratory Medicine, The Ottawa Hospital, the clindamycin susceptibilities of GBS isolates are now reported on the basis of the results of testing with erythromycin. For GAS isolates, the use of erythromycin susceptibility as a surrogate for clindamycin susceptibility may not be appropriate, because approximately 70% of our strains were resistant because of efflux (*mefA*). Therefore, testing of GAS by the double-disk diffusion method would be more appropriate for the reporting of clindamycin resistance.

There is significant geographic variation in the prevalence of macrolide resistance genes, particularly for GAS (11). In southern Ontario, *mefA* accounted for resistance in 91% of the erythromycin-resistant GAS isolates, whereas the rate of resistance accounted for by *mefA* was 62% in this study (12). This may be attributed to differences in the serotypes of the strains circulating in each region (12). In some European studies, the prevalence of *mefA* among erythromycin-resistant GAS isolates has been reported to range from 32 to 64% (6, 8). For

GAS, we did observe differences in the erythromycin resistance rates and the prevalence of the associated mechanism of resistance between adult and pediatric populations. Although the sample size was small, the rate of macrolide resistance was higher among pediatric isolates. Efflux (*mefA*) was the more common mechanism of resistance in both groups of isolates but was more predominant in pediatric isolates. Among the adult isolates, the mechanisms of resistance were more equally distributed between efflux and methylation. For GBS, the variation in resistance mechanisms was not as apparent. The prevalences of inducible and constitutive methylation and efflux in GBS were similar to those previously reported from southern Ontario (4). These differences emphasize the need for laboratories to understand the prevalence of mechanisms of macrolide resistance to determine the most appropriate approach to the reporting of clindamycin susceptibility. Although the results of disk diffusion and MIC testing correlated well with the presence of constitutive MLS_B resistance (*ermB*), only double-disk diffusion testing accurately differentiated efflux (*mefA*) from inducible MLS_B resistance (*ermTR*) for both GAS and GBS (data not shown). We did not determine the optimal separation between the erythromycin and clindamycin disks. Whether the separation obtained with regular disk dispensers would be optimal for the detection of inducible MLS_B resistance, as described for *Staphylococcus* species isolates (9), still needs to be determined. Nevertheless, double-disk diffusion testing remains a simple and reliable alternative method to PCR for deciding how to report clindamycin susceptibility results for GBS and GAS and can easily be incorporated into routine testing.

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