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Macrolide Resistance Determinants of Invasive and Noninvasive Group B Streptococci in a Turkish Hospital

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Macrolide resistance in 156 consecutive group B streptococcal isolates was investigated. Thirty-five isolates (22.4%) had inducible (80%) or constitutive (20%) erythromycin resistance. The genes responsible were *erm*(B), *erm*(A) subclass *erm*(TR), and *erm*(B) plus *erm*(TR) in 62.9, 2.9, and 8.6% of isolates, respectively. Nine isolates (25.7%) harbored neither *mef* nor detectable *erm* genes.

Group B streptococci (GBS), namely, *Streptococcus agalactiae*, are one of the most important causes of neonatal meningitis and sepsis. GBS also cause other infections in pregnant women and the elderly. The treatment of choice for *S. agalactiae* infections is penicillin or its congeners. However, if there is a penicillin allergy or a lack of clinical response, macrolides are the major substitutes. Unfortunately, macrolide resistance among gram-positive cocci has been increasing worldwide. Macrolide resistance in streptococci arises mainly from three mechanisms: active drug efflux controlled by the *mef*(A) gene, modification of the drug target on the rRNA through methylases encoded by *erm* genes, and mutational changes in the target rRNA or protein. The resistance is expressed as either a macrolide-restricted (M) phenotype or inducible and constitutive macrolide-lincosamide-streptogramin B (MLS_B) cross-resistance phenotypes (iMLS and cMLS, respectively). Although there are numerous reports of macrolide resistance mechanisms in *Streptococcus pyogenes* and *Streptococcus pneumoniae*, studies of these mechanisms in *S. agalactiae* are very rare (3, 5, 6, 8, 11–13). In this study, the macrolide resistance determinants of *S. agalactiae* isolates in Turkey were defined for the first time.

We collected a total of 156 *S. agalactiae* isolates from 110 vaginal or vaginoanorectal swabs (from women of reproductive age for another GBS screening study) and 46 urine samples (from patients with urinary infections) via standard methods. To avoid any duplication, only one isolate per patient was included in the study. Identification of the isolates was performed with a commercial latex agglutination kit (Avipath-Strep; Omega Diagnostics, Alloa, Scotland, United Kingdom) in addition to the conventional catalase and CAMP tests. All isolates were initially screened for penicillin and erythromycin resistance by the NCCLS disk diffusion method. Macrolide resistance phenotypes were investigated by a double-disk test (9) with erythromycin and clindamycin disks (Oxoid, Basingstoke, United Kingdom). The MICs of erythromycin and clindamycin were measured by the NCCLS agar dilution method for all the resistant isolates. The susceptibilities of these isolates to tetracycline and other antibiotics routinely used for streptococcal infections (azithromycin, clarithromycin, vancomycin, chloramphenicol, and levofloxacin) were also determined by the NCCLS disk diffusion method. All the erythromycin-resistant isolates were analyzed by PCR for the presence of *mef*(A), *erm*(A), *erm*(B), and *erm*(A) subclass *erm*(TR) gene determinants, as described elsewhere (2, 4). Two reference *Escherichia coli* strains carrying *erm*(A) and *erm*(BP) genes, respectively, and one *S. pyogenes* strain positive for *mef*(A/E) (kindly provided by Helena Seppäla, National Public Health Institute, Turku, Finland) were used as positive PCR controls. An *S. pyogenes* isolate harboring *erm*(TR) as confirmed by PCR and sequence analysis was used as the subclass *erm*(TR) control. For statistical analysis of the results, Fisher's exact test was used and P values of <0.05 were considered significant.

All isolates were susceptible to penicillin, while 23 (26%) vaginal and 12 (20.9%) urinary isolates (total, 35 [22.4%]) ($P = 0.6$) were resistant to erythromycin. None of the resistant isolates had the M phenotype. A total of 28 (80%) isolates (8 urinary and 20 vaginal isolates) expressed the iMLS phenotype, and the remaining 7 (20%; 5 urinary and 2 vaginal isolates) expressed the cMLS phenotype. In nine (25.7%) isolates none of the investigated genes were detected. None of the isolates carried the *mef*(A) or *erm*(A) gene. *erm*(TR) and *erm*(B) coexisted in three (8.6%) vaginal iMLS isolates. This coexistence was confirmed by repeating the PCR tests for subcultures of these three isolates. Separately, *erm*(A) subclass *erm*(TR) and *erm*(B) gene determinants were detected in 1 (2.9%) and 22 (62.9%) isolates, respectively. Corresponding phenotypes and genotypes are shown in Table 1. All isolates resistant to erythromycin were also resistant to azithromycin, clarithromycin, and tetracycline but were susceptible to vancomycin and levofloxacin. The chloramphenicol resistance rate was 44.2% overall, 43% in cMLS isolates, and 46.4% in iMLS isolates. The difference noted between cMLS and iMLS isolates for chloramphenicol resistance was statistically insignificant ($P > 0.05$). MIC test results are summarized in Table 2.

The English-language literature dealing with the macrolide resistance phenotypes and genotypes of *S. agalactiae* is very limited (3, 5, 6, 8, 11–13). In Turkey as well there is no previously published report on this issue. The macrolide resistance

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TABLE 1. Distribution of MLS_B resistance phenotypes according to genotype

Phenotype	No. of isolates with genotype				
	erm(B)	*erm*(TR)	*erm*(B) + *erm*(TR)	Unknown	Total
cMLS	7				7
iMLS	15	1	3	9	28
Total	22	1	3	9	35

rate of our *S. agalactiae* isolates was high, consistent with previous reports of 18% in Ontario, Canada (3); 21.4% in France (6); 16% in North Carolina (11); 14.7% in Spain (12); and 29.7% in Taiwan (13). It should be noted that this rate is much higher than those observed for group A (2.6%) and C and G (6.95%) streptococci from Turkey (1, 7). This could be due to the fact that these bacteria have different localizations: group A, C, and G streptococci colonize or infect mainly the upper respiratory tract, whereas *S. agalactiae* is isolated mainly from the gastrointestinal and genitourinary systems, where MLS_B-resistant bacteria are more abundant and transfer of resistance to *S. agalactiae* is more likely. The most common phenotype that we observed was iMLS, in contrast to prior reports, in which the most frequent phenotype was cMLS (3, 5, 6, 8, 12, 13). It is possible that iMLS may have been misevaluated as cMLS due to zonal overgrowth caused by induction in the case of extended incubation in the double- and/or triple-disk test. Indeed, for almost all our isolates we observed zonal overgrowth in both disk zones, which made the zones very hard to discern. The zonal growth in blunted clindamycin zones was obviously due to self-induction, which may explain the high MIC of clindamycin (128 μg/ml). Note also the high proportion of isolates carrying *erm*(B), a determinant commonly associated with high-level resistance. The M-phenotype prevalence rates reported from other countries are relatively low: 15% in Canada (3), 6 to 7.4% in France (6, 8), and 5 to 9.3% in Spain (5, 12), with the exception of Taiwan, where the M-phenotype prevalence was 37% (13). Interestingly, none of our isolates had the M phenotype. All the resistant isolates had iMLS or cMLS phenotypes; that is, none of the MLS_B drugs can be used to treat the infections caused by these isolates.

erm(B) was the gene most frequently responsible for MLS_B resistance, in agreement with prior studies, except for one from Canada in which *erm*(A) subclass *erm*(TR) was the most prevalent gene (3). All cMLS-expressing isolates had the *erm*(B) gene as expected. Coexistence of the resistance genes (*erm* with *erm* or *erm* with *mef*) in *S. agalactiae* is not uncommon (3, 5, 6, 12). Three of our isolates (8.6%) also harbored *erm*(TR) and *erm*(B) genes together and expressed the iMLS phenotype. Note that we did not detect the *mef* gene in any isolates. The isolates from which we could not amplify any of the resistance genes investigated might have harbored other *erm* genes not investigated or mutants of the investigated genes. Mutations of some ribosomal proteins such as L4 and L22 are other possible resistance mechanisms for the above isolates (10).

TABLE 2. MICs of erythromycin and clindamycin for the erythromycin-resistant isolates

Phenotype, (*n*)	Drug	MIC (μg/ml)		
		MIC_{50}[a]	MIC_{90}[b]	Range
cMLS (7)	Erythromycin	>128	>128	>128
	Clindamycin	>128	>128	>128
iMLS (28)	Erythromycin	16	>128	1–>128
	Clindamycin	2	128	0.06–128

[a] MIC_{50}, MIC at which 50% of the isolates tested are inhibited.
[b] MIC_{90}, MIC at which 90% of the isolates tested are inhibited.

The high rate of tetracycline resistance (100%) that we observed among the erythromycin-resistant *S. agalactiae* isolates was also noted in some previous reports (>80% in Canada, 89.1% in France, 87% in Spain, and 99.2% in Taiwan). However, the mechanisms of this coexistence are yet not clear (5). On the other hand, the chloramphenicol resistance rate that we detected (44.2%) was considerably higher than those previously reported from Taiwan (20.3%) (13) and the United States (1%) (11). This rate was also considerably higher than that detected in Turkish *S. pyogenes* isolates (5.6%), in which tetracycline resistance was 39% (1). However, to asses the statistical significance of erythromycin-chloramphenicol coresistance detected in our isolates, the erythromycin-susceptible isolates should be compared with the resistant ones.

In conclusion, the MLS_B resistance rate is high and accompanied by very high rates of tetracycline and chloramphenicol resistance in Turkish *S. agalactiae* isolates. Vancomycin and levofloxacin are the two reliable substitutes for erythromycin for GBS infections in patients with a penicillin allergy.

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