JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2004, p. 1263–1264 Vol. 42, No. 3
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.3.1263–1264.2004

Resistance of Group B Streptococcus to Selected Antibiotics, Including Erythromycin and Clindamycin

Judith S. Heelan,[1]* Meredith E. Hasenbein,[2] and Alexander J. McAdam[2]

Department of Microbiology, Memorial Hospital of Rhode Island, Pawtucket, and Department of Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island,[1] and Department of Laboratory Medicine, Children's Hospital Boston, Boston, Massachusetts[2]

Received 31 July 2003/Returned for modification 26 September 2003/Accepted 11 November 2003

Resistance of group B streptococcus (GBS) to antibiotics, particularly erythromycin and clindamycin, was studied. Erythromycin resistance was present in 22% of GBS isolates, and these isolates were constitutively resistant, inducibly resistant, or sensitive to clindamycin. Erythromycin and clindamycin MICs were related to the presence of *ermA*, *ermB*, or *mefA* genes.

Group B streptococci (GBS) cause serious, life-threatening infections in the newborn. Mortality of GBS sepsis in neonates is over 50% and is particularly high in preterm infants. Maternal intrapartum prophylaxis for pregnant women colonized with GBS has been recommended for several years (1, 2, 4, 8, 9), since clinical trials showed that the administration of antibiotics to women in labor drastically reduced early-onset invasive GBS infection in the neonate.

The revised Centers for Disease Control and Prevention guidelines issued in 2002 differ from previous guidelines in that universal culture-based screening for vaginal-rectal colonization with GBS is recommended for all pregnant women at 35 to 37 weeks of gestation. These guidelines recommend susceptibility testing to erythromycin and clindamycin on GBS isolates from penicillin-allergic women at risk for anaphylaxis. The goals of this study were to determine the rate of resistance to erythromycin and clindamycin in GBS colonizing pregnant women and to determine the mechanisms of antibiotic resistance present in the bacteria.

Two hundred strains of GBS isolated from vaginal-rectal swabs collected between January 2002 and April 2003 from pregnant women (one isolate per patient) seen in the Family Medicine Department of a teaching community hospital were stored at −70°C until tested. A single swab was used to collect specimens from the lower vagina and rectum. Standard methods were used to isolate and identify *Streptococcus agalactiae* (GBS).

The Kirby-Bauer disk diffusion susceptibility method was performed for penicillin, vancomycin, tetracycline, erythromycin, and clindamycin according to NCCLS guidelines (13). MICs of erythromycin and clindamycin were determined by E test for all isolates resistant or intermediate to erythromycin. The double disk diffusion test for inducible clindamycin resistance was performed on all isolates resistant to erythromycin but susceptible to clindamycin. Erythromycin and clindamycin disks were placed approximately 16 mm apart on the plate. Inducible clindamycin resistance by erythromycin was detected by a blunting of the clindamycin zone closest to the erythromycin disk, giving the appearance of a "D." Detection of the *ermA*, *ermB*, and *mefA* genes was done using PCR with previously published primers (6).

All GBS were susceptible to penicillin and vancomycin, and 30 (15%) were susceptible to tetracycline. Resistance to erythromycin was found in 44 (22%) of the isolates.

Resistance genes were detected in 100% of erythromycin-resistant isolates (Table 1). Twelve isolates (6%) were resistant to both erythromycin and clindamycin. The MICs of erythromycin and clindamycin for eight of these isolates were greater than 256 μg/ml, and all of these had the *ermB* gene detected by PCR. The *ermA* gene was detected in four other isolates that were resistant to erythromycin and clindamycin. The MICs of erythromycin for these *ermA*-positive isolates ranged from 8 to 32 μg/ml (resistant [R]), and those of clindamycin were greater than 256 μg/ml.

Of the 200 isolates, 32 (16%) were resistant to erythromycin but susceptible to clindamycin. Of these isolates, 21 had the *ermA* gene, and all of these had increased clindamycin resistance upon induction with erythromycin as determined by the D test. The erythromycin MICs ranged from 1.5 to 32 μg/ml (R), and the clindamycin MICs were less than 0.5 μg/ml (susceptible [S]).

There were 11 erythromycin-resistant, clindamycin-susceptible isolates which did not have inducible resistance to clindamycin in the D test. All of these isolates had the *mefA* gene. The erythromycin MICs for these isolates ranged from 2 to 8 μg/ml (R), and the clindamycin MICs were less than 0.5 μg/ml (S). One of our isolates was susceptible to erythromycin (MIC, 0.19 μg/ml) but intermediate to clindamycin (MIC, 0.5 μg/ml). No resistance genes were detected in this isolate.

Penicillin or ampicillin remains the drug of choice for intrapartum antibiotic prophylaxis for GBS colonization in pregnant women. Erythromycin and clindamycin are the drugs of choice for women with serious penicillin allergy who are colonized with GBS (5). Mechanisms of resistance to macrolides were first elucidated in group A streptococcus (8). Similar mechanisms were recognized in *Streptococcus pneumoniae* (15) and staphylococci (10). An increase in resistance of GBS to erythromycin has been reported (12). Erythromycin resistance is mediated by two mechanisms: ribosomal methylation or an

* Corresponding author. Mailing address: Department of Microbiology, Memorial Hospital of Rhode Island, 111 Brewster St., Pawtucket, RI 20860. Phone: (401) 729-2839. Fax: (401) 729-2990. E-mail: jheelan@mhri.org.

TABLE 1. Detection of macrolide and lincosamide resistance genes in erythromycin-resistant strains of GBS

Resistance pattern[a]	Total no. of isolates	No. of isolates (mean erythromycin MIC [SD]) with resistance gene		
		ermB	*ermA*	*mefA*
E-R, CC-R	12	8 (>256 [NA])[b]	4 (24.0 [11.3])	0
E-R, CC-S	32			
D test+	21	0	21 (7.3 [8.9])	0
D test−	11	0	0	11 (4.0 [1.7])
Total	44	8 (>256 [NA])	25 (9.9 [11.0])	11 (4.0 [1.7])

[a] E, erythromycin; CC, clindamycin; R, resistant; S, susceptible.
[b] NA, SD not applicable.

efflux pump. Methylation of 23S rRNA by *erm* (erythromycin ribosomal methylase) enzymes blocks binding of macrolides (including erythromycin), lincosamides (including clindamycin), and streptogramin B (MLS) to the 50S ribosomal subunit, leading to drug resistance (3, 10, 11). The methylase enzymes may be expressed constitutively (MLS-cr phenotype) or inducibly (MLS-ir phenotype) by erythromycin (8). In group A streptococcus, the MLS-cr phenotype is strongly associated with the *ermB* gene (6). We and others have shown that the MLS-cr phenotype in GBS is associated with either the *ermA* or *ermB* gene (7). This suggests that the *ermA* gene in some strains of GBS has mutated such that it is expressed constitutively.

In this and other studies, inducible MLS resistance was associated with the *ermA* gene (7). MLS-ir isolates had moderately high erythromycin MICs and low clindamycin MICs in the susceptible category. Reports of treatment failure in staphylococcal infections suggest that use of clindamycin may result in the emergence of constitutively resistant mutants (14). It has been recommended that staphylococci and streptococci with the MLS-ir phenotype not be treated with macrolides or lincosamides (10). MLS-ir strains appear susceptible in vitro to clindamycin by single disk diffusion or by MIC determination (7) but can be detected by PCR for ermA or a positive D test.

The M phenotype is mediated by the *mefA*-encoded, energy-dependent pump, which pumps out macrolides but not lincosamides or streptogramin B (1, 3, 10). M phenotype bacteria are susceptible to lincosamides even in the presence of erythromycin, and so they have a negative D test. All M phenotype GBS in this study had the *mefA* gene. As previously reported (10), MICs for these isolates were moderate in the resistant category to erythromycin and susceptible to clindamycin. These isolates could be safely treated with clindamycin.

The high rate of erythromycin resistance in GBS strongly supports the current Centers for Disease Control and Prevention recommendation that antibiotic susceptibility testing be performed if erythromycin or clindamycin therapy is needed to prevent neonatal GBS infection. The frequency of the MLS-ir isolates and the risk that such organisms may become resistant to clindamycin suggest that laboratories should consider using the D test on GBS which are resistant to erythromycin but susceptible to clindamycin.

REFERENCES

1. **American Academy of Pediatrics, Committee on Infectious Diseases, Committee on Fetus and Newborn.** 1997. Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection by chemoprophylaxis. Pediatrics **99:**489–496.
2. **American College of Obstetricians and Gynecologists, Committee on Obstetric Practice.** 1996. Prevention of early-onset group B streptococcal disease in newborns. Opinion 173. American College of Obstetricians and Gynecologists, Washington, D.C.
3. **Arpin, C., H. Daube, F. Tessier, and C. Quentin.** 1999. Presence of *mefA* and *mefE* genes in *Streptococcus agalactiae*. Antimicrob. Agents Chemother. **43:** 944–946.
4. **Berkowitz, K., J. A. Regan, and E. Greenberg.** 1990. Antibiotic resistance patterns of group B streptococci in pregnant women. J. Clin. Microbiol. **28:**5–7.
5. **Centers for Disease Control and Prevention.** 2002. Prevention of perinatal group B streptococcal disease. Morb. Mortal. Wkly. Rep. **51:**1–22.
6. **De Azavedo, J. C., R. H. Yeung, D. J. Bast, C. L. Duncan, S. B. Borgia, and D. E. Low.** 1999. Prevalence and mechanisms of macrolide resistance in clinical isolates of group A streptococci from Ontario, Canada. Antimicrob. Agents Chemother. **43:**2144–2147.
7. **De Azavedo, J. C., M. McGavin, C. Duncan, D. E. Low, and A. McGeer.** 2001. Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. Antimicrob. Agents Chemother. **45:**3504–3508.
8. **Detcheva, A., R. R. Facklam, and B. Beall.** 2002. Erythromycin-resistant group A streptococcal isolates recovered in Sofia, Bulgaria, from 1995 to 2001. J. Clin. Microbiol. **40:**3831–3834.
9. **Heelan, J., C. Letourneau, and D. Lamarche.** 1995. Detection of group B streptococcus by immunoassay following enrichment in LIM-selective broth medium. Diagn. Microbiol. Infect. Dis. **22:**321–324.
10. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:**482–492.
11. **Livermore, D. M.** 2003. Bacterial resistance: origins, epidemiology, and impact. Clin. Infect. Dis. **36**(Suppl. 1)**:**S11–S23.
12. **Murdoch, D. R., and L. B. Reller.** 2001. Antimicrobial susceptibilities of group B streptococci isolated from patients with invasive disease: 10-year perspective. Antimicrob. Agents Chemother. **45:**3623–3624.
13. **NCCLS.** 2002. Performance standards for antimicrobial susceptibility testing. M100-S12. NCCLS, Wayne, Pa.
14. **Panagea, S., J. D. Perry, and F. K. Gould.** 1999. Should clindamycin be used as treatment of patients with infections caused by erythromycin-resistant staphylococci? J. Antimicrob. Chemother. **44:**581–582.
15. **Waites, K., C. Johnson, B. Gray, K. Edwards, M. Crain, and W. Benjamin.** 2000. Use of clindamycin disks to detect macrolide resistance mediated by *ermB* and *mefE* in *Streptococcus pneumoniae*. J. Clin. Microbiol. **38:**1731–1734.