

Antimicrobial Susceptibilities of Group B Streptococci Isolated between 1992 and 1996 from Patients with Bacteremia or Meningitis

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In vitro testing of 229 group B streptococcal isolates from a variety of patients with invasive infections indicated uniform penicillin G susceptibility. However, 17 (7.4%) isolates were resistant to erythromycin and 8 (3.4%) were resistant to clindamycin. These results support the continued use of penicillin G as the drug of choice for the treatment and prevention of group B streptococcal disease.

Streptococcus agalactiae or group B *Streptococcus* (GBS) accounts for a substantial number of cases of invasive infections in newborn and young infants, pregnant women, and nonpregnant adults who typically have underlying medical conditions (diabetes mellitus, cancer, liver disease, etc.) (5, 23). The considerable mortality and the morbidity associated with both maternal and infant GBS disease has prompted prevention strategies (1, 2, 11, 12, 25). Recently published guidelines to prevent early-onset GBS disease in infants and attendant maternal febrile morbidity recommend the use of maternal penicillin G (or ampicillin) prophylaxis given intravenously during labor (2, 11). This strategy exposes a large number of pregnant women (an estimated 22 to 27% [11]) to penicillin G or, for women with serious penicillin allergies, erythromycin or clindamycin. This elicits concern for the development of resistance to penicillin G, as has been noted previously for other penicillin-susceptible organisms such as *Streptococcus pneumoniae* (15).

Since the first guidelines were published in 1992, some obstetrical care providers in Houston, Texas, have employed maternal intrapartum chemoprophylaxis to prevent early-onset GBS disease (1). Since expanded use of penicillin G can be anticipated with the new guidelines (2, 11), we sought to establish the antimicrobial susceptibility of GBS isolated from a variety of patients with invasive disease including nonpregnant adults treated before widespread use of intrapartum penicillin G. We also investigated possible trends in susceptibility by year of isolation, patient source, and GBS capsular serotype. Finally, with the recent licensure of a new carbapenem, meropenem (Zeneca Pharmaceuticals, Wilmington, Del.), for the treatment of serious infections including meningitis in infants and children, we thought that information regarding its in vitro activity against GBS would provide potentially useful clinical information.

Group B streptococci isolated from the blood or cerebrospinal fluid (CSF) of patients hospitalized between December 1992 and September 1996 at one of four Texas Medical Center hospitals in Houston were studied. A total of 229 isolates were obtained from the following patient groups: (i) neonates with early-onset disease (70), (ii) infants with late-onset disease (67), (iii) pregnant women (47), (iv) nonpregnant adults (39),

and (v) children (6). Strains were identified by the hospital microbiology laboratories by routine methods and confirmed as GBS by latex agglutination assay (Streptex; Murex Biotech Limited, Dartford, England). They then were serotyped by the capillary precipitin method (18) employing rabbit antisera prepared for GBS capsular types I to VII and were stored until being tested at -80°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing 20% glycerol.

Using the agar dilution method and following the guidelines of the National Committee for Clinical Laboratory Standards (21), we determined MICs for penicillin G, ampicillin, cephalothin, cefotaxime, clindamycin, erythromycin, meropenem, gentamicin, rifampin, tetracycline, and vancomycin. All antibiotics, except for meropenem, which was a gift from Zeneca Pharmaceuticals, were purchased from United States Pharmacopoeia (Rockville, Md.).

Frozen isolates of GBS were thawed, inoculated onto Trypticase soy agar containing 5% sheep erythrocytes, and incubated at 35°C overnight. Five colonies were inoculated into 5 ml of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.). After overnight incubation at 35°C , the broth culture was diluted to achieve the turbidity of the 0.5 McFarland standard (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Absorbance at 625 nm was measured with a Spectronic 710 spectrometer (Bausch and Lomb, Rochester, N.Y.). The suspension then was diluted 1:10 to an inoculum of approximately 10^7 CFU per ml.

Mueller-Hinton agar (Becton Dickinson) supplemented with 5% defibrinated sheep erythrocytes was employed for susceptibility testing. Serial twofold dilutions of each antibiotic were incorporated into the agar. By using a multiple replicator device (CMI-Promex, Bridgeport, N.J.), 1 to 2 μl of the inoculum was transferred to the agar, allowed to dry at 24°C , and then incubated at 35°C for 16 to 20 h in ambient atmosphere. The final inoculum of GBS was approximately 10^4 CFU/spot. Colony counts were performed for 6 to 8 isolates on each assay day, both for 2- to 6-h and for overnight incubations. The inocula were identical as were the MIC results for both incubations. Control plates without antibiotic were processed in a similar manner before and after inoculation of each series of plates containing antibiotics. The control strain, *Staphylococcus aureus* ATCC 29213, was included in each test series to assure reproducibility. The MIC was defined as the lowest concentration of antibiotic that completely inhibited bacterial growth.

The susceptibilities of these 229 GBS strains to 11 antibiotics, expressed in micrograms per milliliter as the MIC at which

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TABLE 1. Susceptibility of 229 strains of GBS to 11 antibiotics

Antibiotic	MIC ($\mu\text{g/ml}$)			
	Range	MIC ₅₀	MIC ₉₀	Median
Penicillin G	0.031–0.125	0.062	0.062	0.062
Ampicillin	0.062–0.125	0.062	0.125	0.062
Cephalothin	0.062–0.5	0.25	0.5	0.25
Cefotaxime	0.062–0.125	0.062	0.125	0.062
Clindamycin	0.031–>64	0.031	0.062	0.031
Erythromycin	0.015–>64	0.031	0.062	0.031
Meropenem	0.062	0.062	0.062	0.062
Gentamicin	8–16	8	16	8
Rifampin	0.125–1	0.25	0.5	0.25
Tetracycline	0.5–>128	64	128	64
Vancomycin	0.25–0.5	0.5	0.5	0.5

50% of the isolates were inhibited (MIC₅₀), MIC₉₀, median, and range, are summarized in Table 1. Each strain was susceptible to penicillin G, ampicillin, cefotaxime, and vancomycin. Penicillin G, clindamycin, erythromycin, and meropenem were the most active agents tested, each with an MIC₉₀ of 0.062 $\mu\text{g/ml}$. These drugs were slightly more active than ampicillin and cefotaxime and were more active than cephalothin, rifampin, and vancomycin, all of which had an MIC of 0.5 $\mu\text{g/ml}$. While the median MICs for erythromycin and clindamycin were even lower than that of penicillin G, 17 (7.4%) isolates were resistant (MIC \geq 1 $\mu\text{g/ml}$) to erythromycin and eight (3.4%) were resistant to clindamycin. Four additional isolates were intermediately susceptible to erythromycin, requiring an MIC of 0.5 $\mu\text{g/ml}$. As expected, most strains were resistant (MIC \geq 8 $\mu\text{g/ml}$) or intermediately susceptible (MIC, 4 $\mu\text{g/ml}$) to tetracycline. The MIC of gentamicin for 156 (68%) of the isolates was \geq 16 $\mu\text{g/ml}$.

We then analyzed patterns of susceptibility of GBS to penicillin G and erythromycin by patient group, year of isolation, and serotype. All isolates were susceptible to penicillin G, but 10 (59%) of the erythromycin-resistant strains were isolated either from pregnant women or from neonates with early-onset disease. While this potential trend is of interest, the small number of resistant strains did not permit statistical validation. Regarding year of isolation, no relationship was found for erythromycin resistance, and when the median MICs for isolates were calculated for each antibiotic by year of isolation, no trends were detected either. Finally, no association between GBS serotype and susceptibility to erythromycin or clindamycin was found.

Our findings demonstrate that GBS isolated from a variety of patients with invasive infection remain uniformly susceptible to penicillin G and ampicillin. Although expanded use of intrapartum chemoprophylaxis may promote the development of GBS resistance, we were unable to identify this phenomenon. For all antibiotics tested, the median MICs remained stable from 1992 to 1996. These results confirm those reported by several investigators (3, 6, 8, 13, 16, 17, 19, 20) but refute those of others (9, 24). Perhaps methodological differences explain the minor disparity between the MICs reported here and those reported by others (9, 24).

Clindamycin and erythromycin were somewhat more active against GBS than was penicillin as determined by MIC₅₀. The observation of resistant strains, however, raises the concern for the use of these agents in the prophylaxis or treatment of GBS infection in patients allergic to β -lactams. Berkowitz et al. (8) reported five (3.2%) GBS isolates that were erythromycin resistant. The MIC₉₀ of erythromycin for our strains was lower

than that reported by Persson and Forsgren (22). It is possible that widespread use of erythromycin for the treatment of gynecologic infections may be important in the emergence of some GBS strains that are resistant to macrolides (8, 26).

Based on the MIC₉₀, penicillin G may be preferred to ampicillin for the treatment of GBS infection. The activities of cefotaxime and meropenem also were excellent and comparable to those reported for other expanded-spectrum cephalosporins and carbapenems (7, 13). Although cefotaxime and meropenem are potential alternatives for the empiric treatment of neonates and young infants with suspected meningitis, once GBS has been identified as the causative agent, penicillin G is preferred because of its demonstrated safety and efficacy, narrow spectrum of antimicrobial activity, and lower cost.

The distribution of GBS capsular serotypes is influenced by patient type (neonate versus adult) and site of infection (mucous membranes versus blood or CSF, etc.). We and others (10) have noted a recent change in the serotype distribution of GBS isolates from all patient groups with invasive infection. Baker and Barrett (4) reported in 1974 nearly even proportions of serotypes I, II, and III among GBS isolates from neonates with early-onset bacteremia. Since 1990, however, serotypes I and III predominate, and type V, first recognized in 1985 (14), is an increasingly frequent isolate from neonates and adults (10). The distribution of serotypes among our isolates reflects these contemporary trends. An unexpected finding, however, was that 30% of the erythromycin-resistant strains were type V. Because there is no available information regarding contemporary antimicrobial susceptibility pattern by GBS serotype, the importance of this observation remains speculative. Meanwhile, laboratories should provide routine susceptibility testing of blood and CSF isolates of GBS, especially if there is an apparent prophylaxis or treatment failure with either erythromycin or clindamycin (8).

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