

## Antibiotic Resistance Patterns of Group B Streptococci in Pregnant Women

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Received 3 July 1989/Accepted 2 October 1989

This study examined the antibiotic resistance patterns of group B streptococci (GBS) isolated from gravid women. A total of 156 vaginal and cervical isolates of GBS were examined for resistance to penicillin, ampicillin, clindamycin, cefoxitin, gentamicin, and erythromycin. No resistance to penicillin or ampicillin was found, nor was penicillinase production demonstrated. A high level of resistance to gentamicin was noted (91%). Of the isolates examined, 9, 9.5, and 15.3% exhibited either resistance or intermediate susceptibility to erythromycin, clindamycin, and cefoxitin, respectively. Thirty strains (19%) exhibited a multiple antibiotic resistance pattern. Given the high penicillin and ampicillin treatment failure rates when attempting to eradicate vaginal GBS colonization and our findings of higher and multiple drug resistance patterns of GBS, the selection of an alternative antibiotic regimen is of considerable clinical importance. We recommend that routine reporting of GBS susceptibilities by clinical laboratories be adopted.

Group B streptococci (GBS) are pathogens which have been associated with preterm labor (15, 18, 19), premature rupture of membranes (15, 18), and neonatal sepsis (2). Babies born to women with rectal, vaginal, or urinary tract carriage of GBS are at risk for colonization (1, 5, 6) and infection in the peripartum period. Intrapartum vertical transmission and early onset GBS infection have been prevented (5) by administration of ampicillin to targeted populations thought to be at risk for infant disease (5, 16, 19, 22).

However, even the most selective strategies have exposed large populations to antibiotic therapy. This renewed interest in antibiotic treatment of parturient women to prevent early onset GBS disease and an increased number of recommendations for screening and treatment of other infections such as *Chlamydia trachomatis* (21) during pregnancy have led to concern about the development of antibiotic resistance by GBS. This concern is supported by reports of the emergence of penicillin-tolerant strains of GBS (12), penicillin treatment failures in infected neonates (12), and the observation of strains with multiple beta-lactam resistance phenotypes in bovine populations exposed to heavy antibiotic use (4). Plasmid-mediated drug resistance has also been shown to occur in GBS and may facilitate the development of multidrug resistance (9).

Most previous studies of GBS antibiotic susceptibility patterns were performed at a time when antibiotic use in pregnancy was less widespread. This study was conducted to determine whether changes in GBS antibiotic susceptibility have occurred in the current era of more liberal use of antibiotics among pregnant patients. Antibiotic patterns of susceptibility of GBS isolated from an inner city population of parturient women were determined for penicillin, ampicillin, gentamicin, clindamycin, cefoxitin, and erythromycin. GBS strains were also assayed for penicillinase production.

### MATERIALS AND METHODS

**Bacterial isolates.** Vaginal and cervical isolates of GBS were obtained at 23 to 26 weeks of gestation or at delivery from women who agreed to participate in a larger study of

vaginal infections in pregnancy sponsored by the National Institutes of Health. Women were excluded if they were under age 16, if they were taking insulin, corticosteroids, or tocolytics, or if they had received antibiotics in the 2 weeks prior to enrollment. GBS were identified by coagglutination (13) with group-specific antisera (Streptex; Wellcome). We randomly selected 156 freshly obtained or frozen isolates from 592 available isolates for further study. Forty isolates were fresh, and the remaining 116 were frozen. When frozen isolates were studied, portions were thawed and incubated at 37°C overnight in Todd-Hewitt broth to confirm viability. Freshly obtained and reincubated cultures were then plated onto Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep blood and incubated overnight to obtain pure, viable GBS cultures.

**Testing for susceptibility to antibiotic and for penicillinase production.** A small number of colonies obtained from each Trypticase soy agar-5% sheep blood plate was incubated in Todd-Hewitt broth for 2 h at 37°C to obtain a McFarland level of turbidity of 10<sup>5</sup> and a logarithmic-growth-phase culture. The pH of the Todd-Hewitt broth was adjusted to a range of 6.0 to 6.7 with 0.5 N hydrochloric acid.

One loop of inoculum from each logarithmic-phase culture was plated uniformly onto Trypticase soy agar-5% sheep blood plates with sterile swabs. The Kirby-Bauer disk diffusion method (20) used with BBL paper disks screened for susceptibility to ampicillin, penicillin, clindamycin, cefoxitin, gentamicin, and erythromycin. Zones of inhibition were measured, and the results were interpreted as resistant, intermediate, or susceptible according to the ranges recommended by BBL. Strains identified as intermediate or as resistant to an antibiotic underwent testing for determination of MIC by standard methods (20). The MIC thresholds, above which a strain was considered intermediate in response or resistant to an antibiotic, are those used in standard testing at the Columbia Presbyterian Medical Center and are comparable to those found in the literature (11). Strains resistant to gentamicin alone were not tested for MIC. Penicillinase production was assayed by the chromagen disk method (Cefinase; BBL Laboratories) and penicil-

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TABLE 1. Antibiotic susceptibility patterns of GBS

Antibiotic	No. (%) of isolates with indicated response <sup>a</sup>		
	Susceptible	Intermediate	Resistant
Gentamicin	3 (2.0)	11 (7.0)	142 (91.0)
Ampicillin	156 (100.0)	0 (0)	0 (0)
Penicillin	156 (100.0)	0 (0)	0 (0)
Erythromycin	142 (91.0)	9 (5.8)	5 (3.2)
Clindamycin	141 (90.5)	11 (7.0)	4 (2.5)
Cefoxitin	134 (85.9)	20 (14.1)	2 (1.2)

<sup>a</sup> *n* = 156. MICs were not obtained for strains of GBS that were resistant or intermediate to gentamicin alone. MICs of ampicillin or penicillin are not reported, as no strains were intermediate in susceptibility. MICs of ampicillin were <0.25 µg/ml for both susceptible and resistant strains. MICs of penicillin were <0.06 µg/ml for both susceptible and resistant strains. MICs of erythromycin were <1 µg/ml for susceptible strains, 1 to 2 µg/ml for intermediate strains, and >2 µg/ml for resistant strains. MICs of clindamycin were <0.03 µg/ml for susceptible and intermediate strains and >0.06 µg/ml for resistant strains. MICs of cefoxitin were <1 µg/ml for susceptible strains, 1 to 8 µg/ml for intermediate strains, and >8 µg/ml for resistant strains.

linase-producing *Staphylococcus aureus* as a positive control.

## RESULTS

Among 1,378 women screened antenatally, 25% were colonized with GBS and 18% were colonized at the time of delivery. The percentages of strains resistant, intermediate, or susceptible to each antibiotic are presented in Table 1. All 156 strains tested were susceptible to penicillin and to ampicillin. No strain produced penicillinase. Of the remaining antibiotics tested, gentamicin exhibited the least activity against GBS, with 91% of the strains resistant. Notably, 9.0, 9.5, and 15.3% of the isolates exhibited either resistance or an intermediate response to erythromycin, clindamycin, and cefoxitin, respectively. Thirty strains (19%) were not susceptible to two antibiotics. Sixteen strains (10%) were not susceptible to three antibiotics, and eight (5%) were susceptible only to the penicillins.

## DISCUSSION

The choice of antibiotic to be used for eradication of GBS colonization of the vagina presents a therapeutic dilemma. While penicillins remain the drug of choice for systemic infection caused by GBS, treatment failure rates of up to 50% (6) have been reported when penicillin or ampicillin is used to eradicate GBS from the lower reproductive tract of women.

Our study confirms earlier reports (3, 17) of 100% *in vitro* susceptibility of GBS to penicillin and ampicillin. Efforts to induce penicillin tolerance by the methods of Kim et al. (12) and Horn et al. (10) were ineffective. Furthermore, no strain of GBS was capable of penicillinase production.

*In vitro* susceptibility, while important, is not the only factor determining treatment efficacy. Tissue levels of penicillin, vaginal pH, and concomitant colonization of the vagina with penicillinase-producing organisms are among the factors influencing treatment failure rates.

Alternatively, erythromycin has been used in the treatment of GBS colonization (14; J. A. Regan, unpublished data). In our experience, treatment failures of only 10 to 15% have been encountered after 10 to 14 days of treatment. This efficacy rate coupled with the wider spectrum of activity of erythromycin against lower reproductive tract pathogens, its safety in pregnancy, and its low incidence of gastrointestinal

side effects when administered in the base form make it a logical choice for treatment of GBS.

Of concern in this work is the level of tolerance for erythromycin exhibited among the 156 isolates studied. Of note, five isolates were resistant to erythromycin while nine demonstrated an intermediate susceptibility. The rate of susceptibility to erythromycin is lower than we have previously reported in a study conducted 2 years earlier (8) and that of other earlier reports (17).

In recent years, the use of erythromycin in the treatment of lower reproductive tract infections such as chlamydia (21) has become widespread. It is possible that resistance to erythromycin is being induced in GBS by prior exposure to erythromycin. We are currently addressing the issue of drug-induced resistance in GBS isolates after prolonged exposure to erythromycin in a 6- to 11-week treatment regimen.

Of interest is our finding of 91% resistance of GBS isolates to gentamicin in light of a controversy which has existed concerning the use of gentamicin in selective broth medium as advocated by Baker et al. (1). This medium contains 8 µg of gentamicin sulfate per ml. Baker et al. claimed this was not inhibitory to GBS on the basis of their study of the susceptibility of 244 GBS isolates to gentamicin, in which the median MIC was 25 µg/ml and the range was 12.5 to 100 µg/ml (3). Our finding of 2% susceptible and 7% intermediately susceptible isolates supports the position of Gray et al. (7), who reported the not infrequent occurrence of gentamicin-resistant strains.

Clindamycin and cefoxitin were selected for study in this work because of their widespread use in treatment of obstetrical patients with postpartum endometritis and the widely held clinical opinion that they provide good coverage against GBS. Earlier susceptibility studies indicated greater-than-95% susceptibility to clindamycin and cefoxitin, while in this study only 90 and 86% of strains were susceptible to clindamycin and cefoxitin, respectively.

Multiple antibiotic resistance patterns are documented in this study, with 16 strains exhibiting resistance or an intermediate susceptibility to two or more antibiotics other than gentamicin.

In many laboratories the susceptibilities of GBS are not tested since isolates of GBS, as reconfirmed in this study, are uniformly susceptible to penicillins *in vitro*. High *in vivo* treatment failure rates have led to use of other antibiotics including erythromycin, clindamycin, and cefoxitin for GBS coverage. We suggest that the levels of antibiotic tolerance exhibited by GBS isolates in this study argue in favor of routine susceptibility testing of clinical isolates. This study coupled with significant *in vivo* penicillin treatment failure rates provide evidence of potential nonefficacious treatment regimen choices even when two-drug combinations are used.

To update what is known of GBS antibiotic susceptibility patterns in an era of widespread antibiotic use during pregnancy, we studied the susceptibilities of 156 GBS isolates to penicillin, ampicillin, clindamycin, cefoxitin, gentamicin, and erythromycin and investigated penicillinase production. We found 100% susceptibility to the penicillins and 91% resistance to gentamicin. Patterns of susceptibility to erythromycin, clindamycin, and cefoxitin revealed resistance or intermediate susceptibility at higher rates than have been previously reported.

Since factors other than *in vitro* susceptibility are operational in the efficacy of penicillin in the treatment of the GBS-colonized gravida, the selection of alternative antibiotic treatment is of considerable clinical significance. In light

of our findings of higher and multiple-resistance patterns of GBS to erythromycin, cefoxitin, and clindamycin, we recommend that routine reporting of GBS susceptibilities be adopted to help guide the clinician in the choice of antibiotic therapy.

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