

OBSTETRICS

The effect of intrapartum penicillin on vaginal group B streptococcus colony counts

Anna R. McNanley, MD; J. Christopher Glantz, MD, MPH; Dwight J. Hardy, PhD; David Vicino, BS

OBJECTIVE: The objective of the study was to determine the temporal relationship between intrapartum penicillin-G (PCN-G) and vaginal group B streptococcus (GBS) counts.

STUDY DESIGN: In 50 women with GBS-positive antenatal cultures, intrapartum cultures were collected just before the first PCN-G dose and every 2 hours \times 4 or until delivery. Colony counts were quantified using serial dilution. Results were standardized as percent of initial colony count and analyzed using sequential Wilcoxon tests.

RESULTS: Of 50 subjects with GBS-positive antepartum cultures, 35 (70%) had positive intrapartum vaginal cultures, of which 27 received intrapartum PCN-G. Degree of vaginal colonization varied greatly be-

tween subjects, and counts (percents) were not normally distributed. From the T_0 (time = zero) colony count standardized to 100%, counts fell rapidly to means \pm SE and medians of $18.2 \pm 7.5\%$ and 0.5% at T_2 ($P < .0001$), $2.5 \pm 1.7\%$ and 0.02% at T_4 ($P = .006$), and less than 0.2% and 0.0% at $T_{6 \text{ and } 8}$ ($P = .07$ and $P = .46$, respectively).

CONCLUSION: Vaginal GBS colony counts fall rapidly after intrapartum PCN-G administration, which may partly explain the effectiveness of chemoprophylaxis.

Key words: group B streptococcus, intrapartum, penicillin-G, vaginal colonization

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Early-onset group B streptococcus (GBS) disease in the neonate is an important preventable cause of neonatal morbidity and mortality. With a 4% mortality rate and a 10% risk of neurologic sequelae, prevention strategies are of great importance.^{1,2} In 1996, the Centers for Disease Control and Prevention (CDC) published consensus guidelines for intrapartum chemoprophylaxis against GBS, recommending either a risk-based or screening-based approach³; in 2002, these guidelines were

★ EDITORS' CHOICE ★

revised to endorse only the screening-based approach.¹ Since the adoption of GBS chemoprophylactic strategies, the incidence of early-onset neonatal disease has declined from 1.8 to 0.5 per 1000 live births.¹

Although we know that antibiotic prophylaxis against GBS is effective, we do not know the mechanism by which administration of antibiotics to GBS culture-positive women in labor works. Is it by antibiotic loading of the fetus and amniotic fluid, by eradication of vaginal GBS, or by a combination of both? Penicillins are known to enter both the fetus and amniotic fluid readily^{4,5} and also to decrease vaginal colony counts over periods of weeks to months when measured prenatally and again postpartum.^{6,7}

The effect of penicillin-G (PCN-G) on vaginal GBS colony counts in the hours after antibiotic administration has not been studied. It is known that severity of GBS disease correlates with degree of maternal colonization⁸ and that penicillin and its analogs reach bactericidal concentrations in fetal serum and amniotic fluid within minutes of maternal ad-

ministration.⁹ Despite rapid antibiotic loading of fetal and amniotic fluid compartments within minutes; however, optimal fetal prophylactic benefit takes hours.^{10,11} For these reasons, we postulated that maternal intrapartum PCN-G administration lowers vaginal GBS colony counts over a time course of only a few hours. Our objective was to determine the temporal relationship between intrapartum PCN-G and vaginal GBS counts.

MATERIALS AND METHODS

This study was undertaken in the inpatient obstetrics unit at Strong Memorial Hospital (Rochester, NY). The study received approval from the Perinatal Research Committee and the Institutional Review Board at the University of Rochester. Informed consent was obtained from each subject prior to enrollment. Parturients at our institution are managed in accordance with the CDC guidelines: in the absence of antibiotic allergy, GBS-positive women in labor receive PCN-G every 4 hours until delivery. PCN-allergic women received cefazolin, clindamycin, or vancomycin, depending on degree of allergy and sensitivity test-

From the Departments of Obstetrics and Gynecology (Drs McNanley and Glantz) and Microbiology (Dr Hardy and Mr Vicino), University of Rochester Medical Center, Rochester, NY.

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Reprints: Anna McNanley, MD, URM Box 668, 601 Elmwood Ave, Rochester, NY 14642; anna_mcnanley@urmc.rochester.edu 0002-9378/\$32.00

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ing. Only those women who received penicillin were studied.

Before our main study, we conducted an institutional review board–approved pilot study to assess the adequacy of a technique of serial dilution for quantitating GBS colony counts. Because serial dilution is a quantitative technique not previously described in GBS studies, the method was first assessed to determine its reliability before using it in our main study. The purpose was to estimate whether, in the absence of antibiotics, GBS colony counts were stable over a short time period. Fifty-seven nurses and obstetrics/gynecology residents (most of whom were not pregnant) volunteered as potential subjects for this study, each self-collecting a screening swab from the distal vagina. If this swab was positive for GBS, the subject collected a series of 5 swabs every 2 hours over an 8-hour time period, at 0, 2, 4, 6, and 8 hours. Colony counts were determined via a serial dilution technique described below. Descriptive statistics were calculated and time comparisons made using sequential Wilcoxon signed-rank tests (T_0 (time = zero) vs T_2 (time = 2 hours), T_2 vs T_4 (time = 4 hours), etc.). For study subjects, Friedman's test was not used because of attrition from women delivering over the 8 hour duration; if it were used, only women completing the entire 8 hours could be included.

For the main study, pregnant women in labor were recruited on arrival to the labor floor between April 2005–February 2006. Inclusion criteria were age 18–50 years, positive previous GBS screening culture, receiving intrapartum intravenous PCN-G for GBS prophylaxis, and having the capacity to consent. Subjects were excluded if they had received antibiotics during the previous week or at the time of admission.

For $\alpha = 0.05$ and $\beta = 0.20$ for paired comparisons, we calculated that it would require 52 subjects to demonstrate a 20% decline from early to final colonization. Because of anticipated attrition because of women delivering before the full 8 hours had elapsed and because some women with positive antenatal rectovaginal cultures would have negative intrapartum vaginal cultures, it was further

estimated that at least twice this many subjects would need to be recruited to achieve adequate numbers at time 8 hours. For these reasons, initial approximations were that at least 100 women would be required for adequate power.

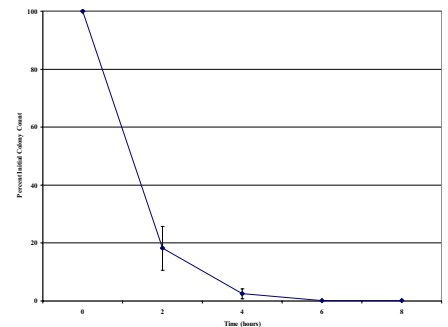
Vaginal cultures were obtained with a modified Stuart's medium swab by trained obstetrics/gynecology residents, nurses, and nurse practitioners. Specimens were taken from the distal third of the vagina at 2-hour intervals (just as in the pilot subjects), starting at time zero just before antibiotic administration, until time equaled 8 hours or delivery, whichever came first. Rectal cultures were not taken, given that previous studies found vaginal colonization to be most important for intrapartum neonatal transmission.^{8,12–16}

Samples were then transported to the microbiology laboratory. Swab specimens were serially diluted in sterile saline by a single laboratory technologist and inoculated onto Columbia Agar containing 5% sheep blood, colistin (10 mg/L), and nalidixic acid (10 mg/L). After incubation for 48 hours at 35°C in ambient air, the number of colonies on the dilution plates were counted visually and multiplied by the appropriate dilution factor for that plate. Clindamycin (2 μ g) and erythromycin (15 μ g) sensitivities were also performed on the first isolate from each subject. To control for initial degree of colonization, results were standardized as percent of initial colony count and analyzed using sequential Wilcoxon tests, with results expressed either as mean \pm SE or median and interquartile range (IQR).

RESULTS

Prevalence of vaginal GBS in our pilot subjects was 7 of 57 (12%). Two of these pilot subjects collected the full 8 hours' worth of cultures on 2 occasions. Distributions of colony counts or percents of baseline were nonGaussian for pilot and study patients. Untreated pilot subjects showed no significant change in vaginal colony counts over 8 hours ($P > .20$ between any 2 consecutive time periods). Of 50 subjects with positive antepartum GBS cultures, 35 (70%) had positive in-

FIGURE
Colony counts rapidly declined following penicillin administration



SEs for both time 6 and time 8 hours were 0.1%, too small to be visible on the chart.

McNanley. Effect of intrapartum penicillin on vaginal group B streptococcus colony counts. *Am J Obstet Gynecol* 2007.

trapartum vaginal cultures, of which 27 (77%) received intrapartum PCN-G. The remainder either received PCN-G but were not included in the study because of the negative intrapartum cultures or received antibiotics other than PCN-G. One culture result was indeterminate secondary to heavy proteus. Three study subjects received 1 dose each of ampicillin and gentamicin for possible chorioamnionitis, all after the third PCN-G dose.

Degree of vaginal colonization varied greatly between study subjects with initial colony counts ranging from 3.0×10^1 to 1.4×10^7 CFU/mL. As illustrated in the Figure from the 27 T_0 colony counts standardized to 100%, subsequently available counts fell rapidly to mean \pm SE and median (IQR) of $18.2 \pm 7.5\%$ and 0.5% (10.6) at T_2 ($n = 25$; $P < .0001$, compared with T_0), $2.5 \pm 1.7\%$ and 0.02% (1.6) at T_4 ($n = 20$; $P = .006$, compared with T_2), $0.1 \pm 0.1\%$ and 0.0% (0.04) at T_6 ($n = 16$; $P = .07$, compared with T_4), and $0.2 \pm 0.1\%$ and 0.0% (0.01) at T_8 ($n = 10$; $P = .46$, compared with T_6). Declining number of subjects in each 2 hour interval reflects women delivering before the 8 hour period was completed (ie, 2 delivered within the first 2 hours, another 5 in the second 2 hours, etc). Women delivering within 4 hours of T_0 received 1 dose of PCN-G, whereas

women delivering after this 4 hour mark received 2 doses.

When the data were stratified by time of rupture of membranes (ROM; ROM before admission vs during the study or unknown), mean initial colony counts (1.7×10^6 vs 1.8×10^6 CFU/mL, respectively) and subsequent declines in percent of initial count were similar to the unstratified data. Using Mann–Whitney *U* tests, there were no statistically significant differences between ROM groups across each time period (all $P > .40$). Mean (median) percents (with initial colony counts again standardized to 100%) for early ROM ($n = 11$) were 100 (100), 6.1 (1.0), 3.8 (0.09), 0.3 (0.001), and 0.2 (0) for times 0-8 hours, respectively ($P < .05$ for T_{0-2} and T_{2-4}). Mean percents for later/unknown ROM ($n = 16$) were 100 (100), 26.3 (0.5), 1.2 (0.01), 0.03 (0), and 0.1 (0) for times 0-8, respectively ($P < .05$ for T_{0-2}).

COMMENT

Serial dilution appears to be a useful technique for determining GBS colony counts and describing response to antibiotic therapy. Using this technique, degree of colonization varies widely between subjects, but in the absence of antibiotics, mean colony counts remain stable over a short period of time. Other investigators have used various techniques for assessing degree of colonization, ranging from time taken to positive identification⁸ to the type of media on which GBS would grow¹⁴ to site of culture positivity.¹⁵ These methods use qualitative or semiquantitative techniques, and our method is the most quantitative of those reported.

As opposed to $\approx 20\%$ population rectovaginal culture positivity,² the 12% GBS yield in pilot subjects in our study is lower because of the absence of rectal culturing. This vaginal yield is consistent with previous studies, which demonstrated 10-13% vaginal GBS positivity.^{16,17}

We found that GBS vaginal colony counts fall rapidly after maternal administration of intravenous PCN-G. This is consistent with high sensitivity of GBS to penicillin. Mean vaginal GBS counts declined 5-fold within 2 hours of antibiot-

ics, and by 4 hours they declined 50-fold from preantibiotic levels. Decline of median colony counts was even more dramatic (200- and 10,000-fold, respectively). This time course is consistent with the findings of de Cueto et al,¹¹ who reported 46% neonatal colonization when intrapartum ampicillin was received less than 1 hour before delivery, declining to 28% neonatal colonization at 2 hours after maternal antibiotics, and between 1% and 3% colonization at 4 hours after antibiotic. Six hours after the first PCN-G dose in our study (2 hours after the second PCN-G dose), mean GBS counts had declined nearly 1000-fold and remained at that level at time 8 hours.

Our findings can be interpreted as PCN-G having a direct effect on vaginal GBS. This may contribute to the benefit of GBS chemoprophylaxis in decreasing the frequency of early-onset GBS disease of the neonate. There is other indirect evidence that lowering colony counts may be helpful. Local application of chlorhexidine, in the absence of maternal systemic antibiotics, decreases vaginal GBS and maternal-neonatal GBS transmission.¹⁸ Local chlorhexidine actually was as effective as ampicillin in 1 study,¹⁹ from which one might conclude that the local effect on colony counts is as important as the beneficial systemic "loading" effect observed in animal studies within 2 hours of maternal antibiotic administration.²⁰ Indirectly supporting this theory is that neonatal prophylaxis immediately following delivery is not as effective as is maternal intrapartum prophylaxis,²¹ although other possible explanations for this would be if infection was established by the time of delivery or if loading alone is not as effective in the absence of concurrently lowering vaginal GBS colony counts.

Because of the limited numbers of women receiving antibiotics other than PCN-G, we did not study the effect of cefazolin, clindamycin, or vancomycin in this study. Because these are used for GBS prophylaxis, they also warrant future study.

A weakness of this study is that multiple vaginal examinations using sterile gloves and lubricated jelly were done.

Unlike KY jelly and Surgilube that contain chlorhexidine, the lubricant used at our institution (lubricating jelly made by Triad Disposables [Brookfield, WI]) does not contain chlorhexidine, although a dilutional effect cannot be ruled out by our data. A significant effect seems unlikely, however, given that there are no reports that multiple vaginal examinations during labor decrease the degree of neonatal GBS colonization. In addition, rupture of membranes, which might be expected to have a greater dilutional effect, did not alter our results.

Although a previous study by Boyer and Gotoff²² examined the effect of intrapartum antibiotics on postpartum rectovaginal cultures, this is the first report to our knowledge that directly addresses the question of intrapartum PCN-G's short-term effect on vaginal GBS colony counts. We observed a greater decline than we had anticipated at the time of our prestudy power calculations, which was fortunate, given that the majority of women delivered before the entire 8 hours of anticipated culturing was complete. Despite this natural attrition of those women enrolling, the decline in GBS counts was dramatic, even at 2 hours, so that the original power calculation of 52 patients was found to be in excess of what ultimately was required. This has potential clinical significance in that women commonly deliver less than 4 hours after the PCN-G loading dose, given that babies born to these women may be kept for prolonged observation, perhaps unnecessarily. We postulate an important role for PCN-G's direct effect as a component of efficacy in GBS prophylaxis. It is uncertain whether this effect is by lowering the likelihood of amniotic fluid infection or by decreasing fetal colonization. ■

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